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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

JULY, 1946

ORIGINAL ARTICLES

THE SPERMATOGENIC ACTIVITY OF Δ^5 -PREGNENOLONE AND OF ITS ESTERS*

BY G. MASSON, PH.D.

MONTREAL, P. Q., CANADA

(From the Institut de Médecine et de Chirurgie expérimentales, Université de Montreal)

Δ^5 -PREGNENOLONE possesses a considerable degree of spermatogenic activity as shown by its ability to prevent the testicular atrophy normally elicited by hypophysectomy,^{1,5,6,7,12} estradiol² or small doses of testosterone.¹⁴ Considered until recently a hypothetic intermediary in the biogenesis of the steroid hormones,¹³ it proved to be a physiologic body constituent since Ruzicka and Prelog¹² isolated it from pig testes. Unlike the most active spermatogenic steroids, pure Δ^5 -pregnenolone does not possess any undesirable action (*e. g.*, Leydig cell atrophy¹⁹), therefore it is likely to become a useful therapeutic agent. In this paper we report on recent investigations concerning the nature of the spermatogenic activity of Δ^5 -pregnenolone and of its acetate, propionate and benzoate. The melting points of the highly purified samples which we used for this work were: free compound 187° to 189° C., acetate 146° to 147° C., propionate 119.5° to 120.5° C. and benzoate 191° to 192° C.

Effects on Hypophysectomized Rats. A first series of experiments was performed on immature albino rats weighing 40 to 60 gm. (average 50 gm.) which were divided into 11 groups of 10 animals each.

Treatment commenced on the day of the operation and was continued for a period of 10 days. At autopsy, the testis and the accessory sex organs were removed, fixed in "Susa," weighed and sectioned. The sella turcica was always carefully examined to ascertain the completeness of the operation. Each compound was given at 2 dose levels, namely 1 and 2 mg. a day dissolved in 0.2 cc. of oil. There were also 3 groups of controls: the first and second consisted of normal untreated rats sacrificed respectively on the 1st and 11th day of the experiment; the third consisted of hypophysectomized untreated animals.

As indicated in Table 1, none of these compounds assured normal testis growth in the absence of the hypophysis. At best they maintained this organ approximately at the degree of development which had been attained at the time of hypophysectomy. None of the esters proved more active than the free compound. That the benzoate appeared least active is probably due to its comparatively slow absorption rate. There was no marked difference between the effects at the 1 mg. and the 2 mg. dose levels.

Similar experiments were performed in

* The expenses of this investigation were defrayed through a grant received from the Frank W. Horner Foundation of Montreal.

The steroids were generously supplied by Dr. Elofson of F. W. Horner Limited, Montreal, and the luteinizing hormone (A. P. L.) by Dr. Stanley Cook of Ayerst, McKenna & Harrison Limited, Montreal.

2 MASSON: SPERMATOGENIC ACTIVITY OF Δ^5 -PREGNENOLONE AND ITS ESTERS

adult albino rats weighing 150 to 180 gm. (average 160 gm.). They were divided into 9 groups (7 experimentals and 2 controls) of 6 animals each. The steroids were either injected at the daily dose level of 2 mg. in 0.2 cc. of oil or implanted in the form of two 10 mg. pellets. The 2 groups of controls consisted respectively of intact and hypophysectomized animals. The results summarized in Table 2 essen-

following experiment was performed in order to determine whether this compound—alone or in combination with gonadotrophic hormones—is able to restore an already atrophic testis to its normal condition. This combined treatment was tried because it is known that a peripheral synergism exists between the gonadotrophic hormones and the folliculoids in the hypophysectomized female.^{11,17}

TABLE 1.—EFFECT OF Δ^5 -PREGNENOLONE ON IMMUNE HYPOPHYSECTOMIZED RATS

Name of compound	Daily dose (mg.)	Wt. of testis (mg.)
Controls non-operated		306 (71)*
Controls non-operated, killed after 10 days		378 (20)
Controls hypophysectomized		91 (6)
Δ^5 -Pregnenolone	1	226 (49)
	2	296 (35)
Δ^5 -Pregnenolone acetate	1	179 (15)
	2	195 (37)
Δ^5 -Pregnenolone propionate	1	214 (52)
	2	188 (28)
Δ^5 -Pregnenolone benzoate	1	166 (31)
	2	121 (6)

* Standard error. The standard errors indicated are calculated by the formula:

$$S.E. = \pm \sqrt{\sum d^2 / n(n-1)}.$$

TABLE 2.—EFFECT OF Δ^5 -PREGNENOLONE ON ADULT HYPOPHYSECTOMIZED RATS

Name of compound	Daily dose (mg.)	Wt. of testis (mg.)
Controls non-operated		2162 (61)*
Controls		7-8 (46)
Δ^5 -Pregnenolone		1600 (14)
Δ^5 -Pregnenolone acetate	2	2051 (120)
Δ^5 -Pregnenolone propionate	2	1747 (49)
Δ^5 -Pregnenolone benzoate	2	1430 (99)
Δ^5 -Pregnenolone		1492 (105)
Δ^5 -Pregnenolone acetate	Pellets	1698 (59)
Δ^5 -Pregnenolone propionate	Pellets	1706 (63)
	Pellets	

* Standard error.

tially confirm our conclusion that testis weight is merely maintained at about the normal level. Perhaps the acetate is slightly more active. Pellet implantation did not maintain the testis as effectively as injections. Histologic examination showed active spermatogenesis in all these hypophysectomized adult treated rats.

To complete our study on the spermatogenic effect of Δ^5 -pregnenolone, the

Adult rats weighing 80 to 100 gm. were hypophysectomized. They were not treated for a period of 14 days so as to permit a severe testis involution. On the 15th day, the animals were divided into 4 groups of 6 animals each and treated for 14 succeeding days as follows: Group I untreated, Group II Δ^5 -pregnenolone (2 mg. a day), Group III Δ^5 -pregnenolone (2 mg. a day) plus luteinizing hormone (100 I.U. a day), and Group IV luteinizing hormone

(100 I.U. a day). On the 15th day of treatment, that is, on the 29th day after hypophysectomy, the animals were sacrificed. The results are expressed in Table 3. From these data it appears that Δ^5 -pregnenolone alone or in combination with the luteinizing hormone is completely ineffective in increasing the testis weight after hypophysectomy. The testis weight is approximately the same in Groups I and II on one side and in Groups III and IV on the other. On histologic examination the germinal epithelium was markedly atrophic in all groups. The Leydig cells were atrophic in Groups I and II while in Groups III and IV they formed large clumps which account for the increase in testis weight over the controls. This interstitial cell stimulation manifests itself furthermore by the marked development of the seminal vesicles and prostate.

Group III estradiol (200 γ a day), Group IV estradiol (2 mg. a day) plus pregnenolone (10 mg. a day), and Group V estradiol (200 γ a day) plus pregnenolone (10 mg. a day). Both steroids were in oil solution. On the 15th day of treatment the animals were killed and the organs taken for weight. The results expressed in Table 4 evidently show that the degree of protection given by Δ^5 -pregnenolone varies with the amount of damage caused by the 2 doses of estradiol. This protecting effect applies to the spermatogenic epithelium as indicated by testis weight as well as to the Leydig cells as indicated by the weight of the seminal vesicles. It has been shown by Selye and Albert¹⁵ that Δ^5 -pregnenolone has no effect on the seminal vesicles of the castrated rat. Incidentally the adrenal stimulation caused

TABLE 3.—EFFECTS OF Δ^5 -PREGNENOLONE AND LUTEINIZING HORMONE ON HYPOPHYSECTOMIZED RATS

Group	Treatment	Daily dose	Testis (mg.)	Seminal vesicles (mg.)	Prostate (mg.)
I	196 (33)*	9 (5)*	14.5 (7)*
II	Δ^5 -Pregnenolone	2 mg.	189 (10)	10.5 (4)	17.5 (7)
III	Δ^5 -Pregnenolone +	2 mg.	326 (21)	134 (16)	101 (8)
	L. H.	100 I.U.			
IV	L.H.	100 I.U.	408 (30)	133 (13)	118 (10)

* Standard error.

Effects on Estradiol Treated Rats. The first experiment is merely a repetition of a previous work made by Albert and Selye.² It was undertaken because at that time a sample of the pregnenolone used by these authors showed a fairly strong folliculoid activity at the daily dose of 3 mg.,¹⁶ while with the present sample, a dose of 50 mg. was necessary to obtain the same result. It is therefore considered possible that impurities might have masked some of the spermatogenic effect of this compound.

Male albino rats weighing 150 to 180 gm. (average 160 gm.) were divided into 4 experimental and 1 control groups of 6 animals each. The treatment was as follows: Group I consisted of untreated controls, Group II estradiol (2 mg. a day),

by estradiol is also slightly diminished by pregnenolone.

In order to study the effect of Δ^5 -pregnenolone on the regeneration of the testis, experiments were performed on rats previously treated with estradiol. Albino rats weighing 150 to 180 gm. (average 160 gm.) were divided into 6 groups of 10 animals each: 5 experimentals and 1 control. Group I received estradiol for 21 days then remained untreated for 21 days; Group II received estradiol for 21 days then Δ^5 -pregnenolone for another period of 21 days; Group III remained untreated for 21 days then received estradiol plus Δ^5 -pregnenolone for another period of 21 days; Group IV received Δ^5 -pregnenolone for 42 days; Group V remained untreated for 21 days then received

estradiol for another period of 21 days; Group IV consisted of untreated controls.

Estradiol was administered in the form of 2 pellets of 10 mg. each per animal which were removed whenever the treatment was discontinued; Δ^5 -pregnenolone was injected at the daily dose of 10 mg. (oil solution at the concentration of 25 mg. per cc.). On the 43rd day of treatment the animals were sacrificed and the tissues were removed for weight and histologic examination.

between Groups IV, V and VI, it is doubtful that the difference is very significant between Groups I, II and III.

In order to evaluate these results it was necessary to repeat a similar experiment using comparatively Δ^5 -pregnenolone and testosterone. For this purpose 5 groups of 10 albino rats weighing 40 to 65 gm. were used. After an initial period of treatment consisting in the implantation of two 10 mg. pellets of estradiol per animal for a period of 14 days,

TABLE 4.—EFFECTS OF Δ^5 -PREGNENOLONE IN RATS TREATED WITH VARIOUS DOSES OF ESTRADIOL

Group	Treatment with daily dose	Testis (mg.)	Seminal vesicles (mg.)	Adrenals (mg.)
I	2455 (97)*	282 (34)*	25 (1.5)*
II	Estradiol, 2 mg.	1124 (103)	77 (11)	48 (5)
III	Estradiol, 200 γ	1416 (126)	87 (9)	42 (3.4)
IV	Estradiol, 2 mg.	1864 (175)	115 (4.8)	43 (2.7)
	+ Pregnenolone, 10 mg.			
V	Estradiol, 200 γ + Pregnenolone, 10 mg.			
		2018 (155)	161 (2.5)	36 (2.9)

* Standard error.

TABLE 5.—EFFECT OF Δ^5 -PREGNENOLONE IN ESTRADIOL TREATED RATS

Groups	Pretreatment	Treatment	Testis (mg.)	Seminal vesicles (mg.)	Adrenals (mg.)	Spermatogenesis from 0 to 4
I	Estradiol	911 (85)*	60 (7.8)*	49 (1.5)*	1.30
II	Estradiol	Pregnenolone	826 (76)	66 (6.6)	45 (2)	1.10
III	Estradiol + Pregnenolone	742 (85)	51 (2.5)	44 (2.7)	0.85
IV	Pregnenolone	Pregnenolone				
V	Estradiol				
VI	2028 (73)	370 (25)	32 (1.5)	4.00
			519 (31)	47 (1.4)	39 (2.8)	0
			2098 (69)	375 (31)	27 (0.7)	4.00

* Standard error.

From Table 5 it appears that at the dose of 10 mg. Δ^5 -pregnenolone (1) has no damaging effect on the testis (compare Groups IV and VI), (2) prevents to a certain degree the testis atrophy produced by estradiol (compare Groups III and V), (3) does not significantly influence the regeneration of testis previously damaged by estradiol under these conditions (compare Groups I and II).

The testes were histologically examined and the degree of spermatogenesis expressed on a scale of 0 to 4. In the table we give the average with, in brackets, the range in the degree of spermatogenesis. While the differences are very striking

the various groups were subsequently treated in the following manner: Group I remained untreated; Group II received pregnenolone (10 mg. a day); Group III received testosterone (10 mg. a day); Group IV received testosterone (2 mg. a day); Group V received pregnenolone (10 mg. a day) plus testosterone (2 mg. a day). On the 29th day after pellet implantation the animals were killed. The results are summarized in Table 6. It is obvious that a marked difference exists between the activity of Δ^5 -pregnenolone and that of testosterone. Testosterone at the 2 mg. dose level does not influence to any great extent the regenera-

tion of the testis, while at the 10 mg. dose level it accelerates it markedly. On the contrary pregnenolone at the dose of 10 mg. maintains the testis in atrophic state by inhibiting the regeneration process. However, it is interesting to note that the seminal vesicles are perhaps more stimulated in Group II which received pregnenolone than in Group I which remained untreated. This would suggest that pregnenolone which is not testoid has stimulated the secretion of endogenous testoid hormone.

tomy, treatment with estradiol, small doses of testosterone or non-specific agents. The observations of Nelson,^{8,9,10} Cutuly,^{3,4} Selye and Friedman¹⁸ and Masson⁷ showed that these steroids can be divided into 2 groups: those which are capable of restoring spermatogenesis in a testis previously rendered atrophic by hypophysectomy (compounds of the testosterone and androstenediol type), and those which do not possess this property (compounds of the progesterone type). The experiments reported in this communication support

TABLE 6.—EFFECT OF Δ^5 -PREGNENOLONE AND TESTOSTERONE ON RATS PREVIOUSLY TREATED WITH ESTRADIOL

Group	Treatment with daily dose	Testis (mg.)	Seminal vesicles (mg.)
I	527 (65)*	49 (4)*
II	Δ^5 -Pregnenolone, 10 mg.	237 (15)	61 (6)
III	Testosterone, 10 mg.	888 (71)	822 (51)
IV	Testosterone, 2 mg.	410 (71)	246 (10)
V	Δ^5 -Pregnenolone, 10 mg. + Testosterone, 2 mg.	344 (21)	204 (10)

* Standard error.

Due to the significance of these results from the point of view of the mode of action of the spermatogenic steroids, it seemed to the author that before discussing such a possibility, another experiment should be undertaken. Rats weighing 85 to 95 gm. were used and divided into 4 groups pretreated for the same length of time with estradiol. The treatment was as follows: Group I untreated controls, Group II pregnenolone (10 mg. a day), Group III testosterone (5 mg. a day) plus pregnenolone (10 mg. a day) and Group IV testosterone (5 mg. a day). The animals were killed after 10 days of treatment. The average testis weight was respectively 810, 261, 583 and 547 mg. for each of the successive groups. These data, therefore, confirm the previous conclusions concerning a difference in the way of action between testosterone and pregnenolone.

Summary and Conclusions. By spermatogenic activity of steroid compounds, we understand their ability to prevent testicular atrophy following hypophysec-

the value of this classification. We have shown that testosterone accelerates, while Δ^5 -pregnenolone inhibits the regeneration of the atrophic testis. One can say that testosterone is an *active* spermatogenic compound which directly stimulates the proliferation of the germinal epithelium, while Δ^5 -pregnenolone is a *passive* spermatogenic agent, whose action consists merely in protecting and maintaining the testis in the condition in which it is at the commencement of treatment.

This classification may serve as a basis for the rational selection of gonadotrophic steroids for clinical use. The steroids which belong to the testosterone and androstenediol type should only be used when the testis is already damaged because in the case of almost normal testes, there is a danger of causing Leydig cell atrophy by such treatment and this may represent a greater evil than the diminution in the spermatogenesis. Conversely Δ^5 -pregnenolone should only be used if a comparatively normal testis is to be pro-

6 MASSON: SPERMATOGENIC ACTIVITY OF Δ^5 -PREGNENOLONE AND ITS ESTERS

tected against subsequent damage. With this compound there is no reason to fear any unpleasant side-effects since at the daily dose of 10 mg. it is practically devoid of folliculoid, testoid and corticoid activities and possesses only weak luteoid properties.⁶ These conclusions are in

accord with those of Selye and Albert¹⁴ who suggested that "by combining moderate doses of testosterone with a non-testoid compound such as pregnenolone, therapeutic doses of testosterone may be given in cases of eunuchoidism without the danger of producing testis atrophy."

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TESTOSTERONE IN ANGINA PECTORIS

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THE problem of treatment of angina pectoris continues to vex the internist, and while nitroglycerin remains our most effective agent in relieving each attack as it occurs, the search for a medication which will eliminate or greatly reduce the frequency of attacks in those affected by the disease continues.

Testosterone preparations have been recommended, and recently the use of testosterone propionate injections was brought to popular attention by an article in the lay press and a book by a popular writer. Interest in the value of this drug, which is rather expensive and requires a somewhat tedious course of injections, continues high.

Review of the literature reveals that in 1940 Walker¹⁵ first reported definite subjective improvement, increased emotional and exercise tolerance, decreased severity of pain and general increase in strength in 4 of 5 patients suffering from angina pectoris. In a later report the same author obtained improvement in 7 of 9 men and 2 of 3 women, but 1 of the men had a coronary occlusion during the course of treatment.

Lesser,^{5,6} in 2 enthusiastic articles, claimed excellent results in a total of 41 men and 5 women, treated with testosterone propionate, 25 mg. intramuscularly every 2 to 5 days for 5 to 25 doses, with an average of 11 doses. Improvement appeared in 4 to 6 weeks and was well sustained. Hamm² also reported favorably.

Sigler and Tulgan,¹¹ treating 20 cases which had been demonstrably relieved by nitroglycerin, noted marked relief in 11 and no relief in 4. Strong and Wallace,¹³ in 1944, reporting 20 cases, obtained marked relief in 6 and slight to moderate, transient

improvement in 11, with no relief in 3, and concluded that hormones have some value in treatment of angina pectoris.

Goldman and Markham¹ treated 7 cases of effort syndrome which they considered a complication of the male climacterium, and noted favorable response in 6. The seventh which they diagnosed as true cardiac angina, did not respond. McGavack⁹ treated 8 similar cases not responding to nitrites and observed prompt and dramatic relief from 24 to 48 hours. He suggested that this response be used as a diagnostic test to differentiate functional "climacteric" angina from true "organic" angina. He stated further that the pains of organic heart disease have never responded rapidly and completely, if at all, to testosterone. He felt that the testosterone orally was as effective as testosterone parenterally administered.

Levine and Likoff,⁷ in 1943, analyzed 19 cases of definite angina pectoris, 3 of whom had coronary occlusion, treated with 25 mg. of testosterone propionate intramuscularly 3 times a week for 4 to 7 weeks. Marked temporary improvement was noted in 5 cases but there was no change in 11 cases. These authors were unable to conclude that testosterone was of any benefit, considering the vagaries of the disease. Hurxthal⁴ reported equivocal results with oral and intramuscular administration in 19 cases, and disappointing results from pellet implantation. Riesenman¹⁰ classified testosterone among agents of no value in treatment of angina pectoris.

Waldman,¹⁴ in November 1945, endorsed testosterone as a treatment for true angina pectoris on the basis of his experience in 10 cases, and what he presents as objective electrocardiographic evidence of

increased exercise tolerance. This evidence is not convincing.

The conflicting reports suggested to us that testosterone preparations had value in some condition which, if not true angina, was similar enough in its clinical manifestations to lead to considerable confusion.

Obviously, not all recurrent chest pain is angina and not all precordial pain is angina. The pain of angina is characteristically retrosternal and not precordial. A syndrome has been described due purportedly to failure of gonadal function in the male, occurring usually after the age of 30, but as early as 25 years of age, and termed the male climacteric. Symptoms of this condition include fatigability, palpitation, weakness, dyspnea, abdominal pain and nervousness. We have noted that a feeling of tightness in the chest and of precordial or rarely substernal aching or fullness might also be present, suggesting angina pectoris. This has been previously described.⁹ Considerable concern over the possibility of heart disease was often apparent, and in many instances the true retrosternal pressure pain, choking and vasomotor phenomena of angina pectoris were present in addition to and apart from the less specific symptoms of male climacteric. The complaints of impotence of rather recent development, in conjunction with hot flashes and the other symptoms attributed to the male climacteric were accepted as identifying a particular syndrome, whether or not symptoms of true angina pectoris, were also present. Thus, 2 clinical entities, true angina pectoris, and a somewhat different chest discomfort related to the male climacteric came immediately under observation; both syndromes were coexistent in several patients. The question of whether the symptoms attributed to the male climacteric were altogether due to it, or due in part to anxiety neurosis or so-called neurocirculatory asthenia did not seem to require consideration in the present connection.

We set out to answer the following questions:

1. Does testosterone propionate by injection or methyl testosterone by sublingual administration have any value in treatment of true clinically diagnosable angina pectoris?

2. Do either of these preparations have a field of usefulness in relieving the chest discomfort associated with male climacteric or nervous instability occasionally encountered in individuals of the age group commonly subject to angina?

3. Is there any difference in the effect of the drug depending on the route of administration, whether parenteral or sublingual?

Materials and Methods. Twenty-four male patients, age 33 to 76 years (average 54 years), all but 1 of whom had been referred to us with the provisional diagnosis of angina pectoris, were studied. They had had symptoms from 2 to 72 months before treatment was begun, with an average duration of illness of 16 months prior to treatment. Electrocardiograms were obtained in all cases and Roentgen ray study of the chest performed when indicated.

On the basis of the clinical and electrocardiographic findings, patients were classified by diagnoses into 3 main groups as follows:

Group 1. True angina pectoris—21 patients.

Group 2. Male climacteric with chest discomfort but not true angina pectoris—2 patients.

Group 3. Male climacteric symptoms alone—1 patient.

Three subgroups appeared. These were:

Subgroup 1. Symptoms of male climacteric in addition to true angina pectoris—10 patients.

Subgroup 2. Cardiac neurosis with true angina pectoris—2 patients.

Subgroup 3. Cardiac neurosis with climacteric syndrome—1 patient.

Valvular heart disease was present in 2 cases of the series, due in both cases to rheumatic heart disease. In 1 aortic stenosis and insufficiency and mitral stenosis

were present and in the other minimal aortic stenosis alone.

Hypertension, with blood pressure consistently greater than 140/90 was present in 6 cases.

Four patients had had definite coronary occlusion proved by history and electrocardiographic changes before the treatment was instituted, and 4 other patients may have had old myocardial infarction, although their histories were not conclusive and their electrocardiograms were only suggestive.

glycerin required. (3) The response to the exercise tolerance test of Master.¹² This test was applied in only 7 cases and no change was apparent as a result of treatment in any. The test was not used more extensively because of the difficulty of maintaining the same conditions as to room temperature, physical status of the patient and time of day, all of which may influence the result and make it difficult to interpret.

Results with reference to the symptoms of male climacteric or of cardiac neurosis

TABLE 1.—RESULTS IN RELATION TO DIAGNOSTIC CATEGORY

No. patients	Diagnostic category	Results				Death	Total courses
		No improvement	Slight improvement	Moderate improvement	Marked improvement		
21	True angina pectoris	11	4	4	2*†	1	22
2	Climacteric with chest discomfort (not angina pectoris):						
	Climacteric (general)	2‡§	}	2
	Chest discomfort	2‡§		
1	Climacteric	1	..	1
10	Climacteric symptoms with true angina pectoris:						
	Climacteric	2¶			6	1	}
	Angina	5	2	1	1	1	
2	Cardiac neurosis with true angina pectoris	1	1†	..	2
1	Cardiac neurosis and climacteric	1§	..	1

* 1 patient was 75% improved.

† 1 patient had a large psychic element. Said he "felt like a new man" after the third injection.

‡ All symptoms helped by 1 injection (1 case).

§ Relief after 4 weeks' treatment, Schedule C (alcoholic with severe cardiac neurosis).

¶ No record kept as to effect of treatment on climacteric symptoms in 1 additional case.

Two routes of administration were used, intramuscular and sublingual. Twenty-five courses were given, 1 patient receiving both sublingual and intramuscular medication. The dosage schedules are indicated in Table 2.

Results with reference to the anginal symptoms were evaluated and classified as: No Response, Slight, Moderate, or Marked Improvement. There was 1 death after treatment was begun. Evaluation was based on: (1) Questioning the patient as to his subjective response. (2) Questioning the patient as to the amount of nitro-

were classified simply as: Relieved or Not Relieved, on the basis of the patient's replies to questioning.

Results. The results we obtained are summarized in the following tables:

Table 1 shows the results of treatment according to diagnostic category.

Of the 21 patients with true angina pectoris who received a total of 22 courses of treatment, 11 showed no improvement, 4 showed slight, 4 moderate, and 2 marked benefit. One died of coronary occlusion 72 hours after treatment was begun. The occlusion occurred after 3 doses of methyl

testosterone had been taken (see Table 2). Of the 2 patients with marked improvement, 1 was considered about 75% relieved, but was not cured, and 1, who showed a large psychogenic factor and was classified also in the group of cardiac neurosis with true angina said he "felt like a new man" after the third injection. These results are not startling and hardly justify any claim that these drugs in the dosage employed are of real benefit in true angina pectoris.

5 cases, slightly improved in 2, moderately improved in 1, and markedly improved in 1. One patient, previously mentioned, died shortly after treatment was instituted. Again the lack of parallelism between the responses of the 2 groups of symptoms is demonstrated, and the lack of value of testosterone in treatment of angina is evident.

Two patients with significant cardiac neurosis and true angina pectoris were treated. One was not helped and the

TABLE 2.—RESULTS IN RELATION TO DOSAGE SCHEDULE AND ROUTE OF ADMINISTRATION

Schedule	Total No. courses	Angina pectoris (21 patients)				Climacteric (13 patients)			
		No response	Slight improvement	Moderate improvement	Marked improvement	Died	Relieved	Not relieved	Died
A	1	1							
B	3	1	1	1	2		
C	7	1	1	2	1	..	3		
D	5	2	1	1	1	..	1*		
E	8	6	1	1	4	
E'	1	Patient had coronary occlusion 24 hours after treatment started and died 48 hours later				1	1

* Effect on symptoms of climacteric not recorded in 1 additional case.

Schedule	Total weekly dosage (mg.)
A. 25 mg. testosterone propionate intramuscularly once weekly for 6 weeks	25
B. 25 mg. testosterone propionate intramuscularly twice weekly for 6 weeks	50
C. 25 mg. testosterone propionate intramuscularly 3 times weekly for 4 weeks	75
D. 5 mg. methyl testosterone sublingually twice daily for 6 weeks	70
E. 5 mg. methyl testosterone sublingually 3 times daily for 4 weeks	105

The 2 patients with climacteric symptoms associated with chest discomfort consisting of precordial pain or oppression, dyspnea and left mammary pain were both markedly benefited with regard to both general symptoms and chest discomfort. Although the number is small, the drug seems to have considerable value here.

One patient with climacteric symptoms alone was greatly helped.

In the group of patients with angina were 10 whose angina was complicated by symptoms of male climacteric. The climacteric symptoms were unrelieved in 2 of these. There was no record kept of results in 1, and relief was obtained in 6. The symptoms of angina did not react in a similar fashion, but were unrelieved in

other patient, previously mentioned, in whom the psychogenic factor was hard to evaluate accurately but in whom it was felt true angina was present, was relieved.

One patient with severe cardiac neurosis and precordial discomfort associated with the climacterium was relieved.

Table 2 shows the influence of the route of administration and dosage on the effectiveness of the drug.

With regard to the symptoms of angina pectoris, it apparently made little difference whether the drug was given sublingually or parenterally as little benefit was obtained anyway.

The climacteric symptoms were more uniformly relieved by parenteral administration of testosterone propionate than by

sublingual administration of methyl testosterone in the dosage employed.

A note regarding dosage of the drug for sublingual use is in place here. The weekly total dose is shown in the table. The effective dose of testosterone given orally is supposed to be 3 or 4 times the intramuscular dose.^{12,16} The effective intramuscular dose in our experience was 50 to 75 mg. per week. The expected effective oral dose would therefore be 150 to 300 mg. per week. The dosage of methyl testosterone linquets for sublingual administration is supposed to be from one-half to two-thirds of the calculated oral dosage.^{8,12} Thus an effective dose might be expected to fall between 75 and 200 mg. each week. Our patients received either 70 or 105 mg. each week. These dosage schedules were chosen for the sake of convenience, and because it appeared from studying other reports that they should provide sufficient medication. In this connection it is noteworthy that Heller and Myers,³ in treating the male climacteric, found sublingual methyl testosterone unsuitable because it was ineffective or produced undesirable side reactions, such as burning of the mouth, swelling of the gums, nausea, vomiting, heartburn, weakness of the legs, tinnitus, vertigo and

headache. In 2 of our cases, untoward symptoms were associated with sublingual administration, 1 patient claiming that methyl testosterone kept him awake and the other that it caused indigestion. Both felt that the attacks of angina increased in frequency while taking the drug.

Conclusions. Although this study deals with a small and statistically insufficient group of cases, the indications seem fairly clear.

The questions posed earlier may now be answered.

1. Neither testosterone propionate intramuscularly nor methyl testosterone sublingually appears to have any value in the treatment of angina pectoris.

2. Testosterone preparations appear to be of definite value in relieving the chest discomfort sometimes associated with the male climacterium or the similar precordial ache of neurocirculatory asthenia occasionally encountered in individuals in the age group commonly subject to angina pectoris.

3. In their field of usefulness, parenteral administration of 25 mg. of testosterone propionate 2, or preferably 3, times weekly is to be preferred to the rather ineffective administration of methyl testosterone sublingually in doses of 10 or 15 mg. daily.

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ECHINOCOCCOSIS IN ICELAND

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HYDATID cysts have during the last centuries been of frequent occurrence in Iceland. Reliable statistics are not available, but Schleisner¹⁰ estimated that 1 out of 7 persons suffered from this disease about the middle of the 19th century. Of 2600 patients of all ages which Icelandic physicians had examined at that period, according to official medical reports 1 out of every 8 had liver disease. But of Schleisner's 326 patients, 1 out of every 6 had hydatid cysts. However, Magnússon⁹ found many faults with Schleisner's calculations, regarding these estimates as far too high.

Schleisner's estimates have been cited in the medical literature, and up to the present they have been the basis of information upon which the medical world has depended with regard to this disease in Iceland.

A little later Finsen⁶ found 298 patients with hydatid cysts out of a total number of 7044, or 1 of every 22.6. This author thought this number proportionally too high, as many patients with this disease came to him from outside his district. By counting hydatid patients in families of his personal acquaintance he found 13 patients in 46 families with a total of 561 members, or 1 in 43. Finsen inclined to conclude that 1 of every 40 to 50 inhabitants in this country harbored hydatid cysts.

In a monograph on hydatid disease Jonassen⁷ criticized Finsen's calculations, chiefly on the ground that Finsen had not seen the majority of his patients and had only operated on 50 of 298. Jonassen tried to form an estimate by inquiring among 13 medical officials whom he regarded as trustworthy and obtaining reports from them on all known patients with hydatid cysts in their districts, where

the total number of inhabitants was known.

The result of this inquiry was that out of 9982 individuals 122 were found with clinically manifest hydatid cysts or 1:82. Jonassen considered this a low estimate, because Reykjavik was included, where he found only 1 patient among the 2000 inhabitants. By omitting Reykjavik the ratio was brought up to 1:61, which he considered a reasonable estimate for the whole country.

The only available autopsy reports previous to the one here presented are from the leprosy hospital outside Reykjavik where Bjarnhéðinsson⁴ found hydatid cysts in 26 out of 86 autopsies, or in 30%. G. Magnússon, however, thought these findings not representative for the whole population, as the lepers were from homes which might be expected to have a higher rate of echinococcosis than the average. Magnússon⁹ tried to make an estimate of the infestation rate of the entire country, taking Jonassen's calculations as a basis for his own, which were made 30 years later (1914). By comparing the number of his patients with hydatid cysts with those of Jonassen's time, he found the morbidity rate only one-fourth of what it had been in 1882. Magnússon's monography (written in 1913) concluded: "There should be not more than 300 patients in the whole country, and their number is steadily decreasing, as the dead patients outnumber the fresh crops."

Hydatid Cysts Found at Autopsies. 1930-1944 (incl.). In this 15 year period, 1231 autopsies were performed. In this number, stillborn children and those under 14 days old have been omitted. On the other hand, 2 cases are included in whom no parasites were found at the autopsy,

but who had been operated on and the parasites removed.

Table 1 shows the number of autopsies performed each year and the number of individuals harboring hydatid cysts. According to this survey, a total of 60 individuals were infested with echinococcus granulosus or showed unmistakable signs of having been infested. This corresponds to 1 echinococcus carrier out of every 20.5.

TABLE 1.—NUMBER OF AUTOPSIES AGAINST ECHINOCOCCUS CASES, 1932-1944

1932 . . .	32:5	15	6%
1933 . . .	74:4	5	4%
1934 . . .	71:4	5	6%
1935 . . .	96:3	3	1%
1936 . . .	91:1	1	1%
1937 . . .	118:6	5	1%
1938 . . .	115:2	1	6%
1939 . . .	88:7	8	0%
1940 . . .	98:4	4	0%
1941 . . .	115:6	5	2%
1942 . . .	121:7	5	8%
1943 . . .	84:3	3	6%
1944 . . .	125:8	6	4%

TABLE 2.—ECHINOCOCCOSIS FOUND IN VARIOUS AGE GROUPS (34 MALES, 26 FEMALES)

Age	Total autopsies	Echino-coccus cases	%
0-20 . . .	239	0	0
21-30 . . .	193	1	0 5
31-40 . . .	199	1	0 5
41-50 . . .	170	4	2 4
51-60 . . .	179	13	7 3
61-70 . . .	153	21	13 7
71-90 . . .	98	20	20 0
	1231	60	4 9

Table 2 shows that hydatid cysts are chiefly found in the higher age groups, but are comparatively rare among the young, no cyst being found in anyone under 20 years of age. In those over 70 years of age hydatid cysts were found in 20 (20%), but in all persons past 60, numbering 151, hydatid cysts were found in 41 (16%). In those past 60 years of age 1 of every 6 harbored 1 or more echinococcus cysts.

These are the people who in Magnússon's time were in their prime of life. As there is no reason to assume that echinococcus carriers have been spared in preference to other people, the conclusion is justified that in 1913 every 6th person

above 30 years of age has been infested with echinococcus.

All the authors mentioned above, who have estimated the distribution of echinococcosis, have based their figures on the number of echinococcus patients, *i. e.*, individuals who have had inconveniences from their hydatid cysts, sufficient to classify them as patients. Obviously no clear-cut line can be drawn between healthy and sick persons in this respect. This method of previous authors was only dictated by necessity, when no other way was possible to discover the actual percentage of infestation and all authors realized the lack of exactness in their estimates, pointing out that reliable information will not be obtained until autopsy reports are available.

Localization. By far the most of the parasites were localized in the liver (56 out of the total of 60). In 4 cases hydatid cysts were found in the peritoneum; in a 59 year old female 2 cherry-sized calcified parasites were found in the omentum. A 62 year old male had a parasite of hen's egg size adherent between the ileum and the cecum, and in a 75 year old male a parasite of similar size was found on the right side of the urinary bladder in the pelvis. In the 4th case numerous secondary cysts up to walnut size were scattered all over the peritoneum. In all these cases one or more cysts were found in the liver.

Other locations were very rare: 2 in the spleen and 2 in the heart, but no hydatid cysts were found in other organs.

Our experience corroborates Claessen's² findings, that primary echinococcus cysts in the lungs do not belong to the picture of echinococcosis in this country. We found no hydatid cyst in the lungs in our material.

This distribution is a little different from Jonassen's who found 18% of his material in the kidneys. Of 50 hydatid cysts, 9 were in the kidneys, 3 in the spleen and 1 in the heart. The distribution has therefore been similar with exception of the kidney cysts.

We have a strong suspicion that at least some of Jonassen's kidney cysts were not echinococcus cysts, but only urinary retention cysts which are fairly frequent and may reach considerable size, sufficient to mislead unexperienced investigators. Jonassen evidently has realized that his kidney cases were not reliable, for by sifting his material critically he finally counted only 2 of the 9 as beyond doubt. Certainly 1 of these must have been an echinococcus, because daughter cysts were found in it.

It is worth notice that 2 of the cysts in our material were found in the heart. Probably this localization is not so rare as commonly supposed; it seems more than co-incidental that we found 2 such in a total of 60. One of them was a bladder of cherry size, loosely adherent to the endocardium of the right ventricle. This cyst was found in a 26 year old male from the country who died of phthisis. The parasite was alive and evidently very young, probably not over 2 to 3 years old (the patient had been in a tuberculosis hospital during the previous 2 years, where he could not have an opportunity of infesting himself). The other case was located in the septum ventriculorum and had ruptured into the pericardium. This case has been described elsewhere by us.⁵

Liver Cysts. Of the 56 liver cysts the localization is not mentioned in 8 cases. In the 48 cases in which the localizations are recorded, the parasites were distributed as follows: right lobe, 32 (67%); left lobe, 6 (12.5%); both lobes, 6 (12.5%); ligamentum falciforme or intermediary zone, 4 (8%).

Other investigators (Dew⁴) have found parasites in both lobes only in 2 to 4% of their cases. Our findings correspond with the heavier infestation in this country. All investigators seem to find the parasites 5 times more frequently in the right than in the left lobe.

Cases of Special Interest. CASE 1. No. 67-42. A 47 year old male was brought from the country with the diagnosis subphrenic abscess, but his condition was so

bad that operation was not considered advisable, and he died a few days later.

In the pleural sac there were 2500 cc. of purulent fluid. The right lung was completely collapsed, lying as a thin sheet close to the mediastinum and the diaphragm. The lung was adherent to the diaphragm, but close to its border was a perforation through which purulent material passed from below the diaphragm. When the lung was torn from the diaphragm a round opening, 8 cm. in diameter, was discovered in the diaphragm, leading directly into a hydatid cyst of cocoanut size. The adventitia is slightly calcified. The contents are a gelatinous mass with a few collapsed daughter cysts. The cyst is adjacent to the vertebral column and touches the superior surface of the right kidney. No other hydatid cysts were present.

CASE 2. No. 11-1944. In a 59 year old male, who had been operated on for echinococcus 13 years before, symptoms of prostatic hypertrophy and uremia led to death in 3 months.

At *autopsy*, the liver weighed 1750 gm. When the abdomen was opened, an abscess of fist size was opened close to the sternum, below the right curvature. Rests of chitinous membranes pointed to the hydatid origin of the suppuration. The right lobe was malformed by hydatid cysts. One prominent cyst had grown tower-like upwards from the liver on the right side and into the lung, compressing the diaphragm before it, in such a way that it could not be distinguished from the adventitia of the capsule. This cyst was very slightly calcified and contained numerous daughter cysts and foul purulent fluid. The same condition was found in a hydatid cyst close by. The volume of each of these cysts corresponded to that of an apple, the elongated one being a little larger and of oblong shape.

In this case the capsule still protected the surrounding lung tissue, but the cyst was evidently on its way to suppuration into the lung.

CASE 3. No. 63-44. A 51 year old female who had for 14 years been in a lunatic asylum, developed a distention of the abdomen which was thought to be caused by an ovarian cyst. Three days before death she vomited blackish, turbid fluid and had severe pains in the abdomen. Prostration

and collapse followed quickly and led to death.

When the abdomen was opened a huge echinococcus cyst was discovered, which was attached to the right lobe of the liver. The intestines were in several places adherent to the cyst, particularly the transverse colon, the anterior surface of the stomach, duodenum and the descending colon. The diameter of this cyst was 50 cm. and it contained 16 liters of thick, brownish fluid, with clear uncollapsed daughter cysts. From the left inferior surface of the liver another cyst of hen's egg size was suspended. Above the right kidney were 3 hydatid cysts, all together the size of an apple.

This cyst is probably the largest on record, the biggest reported by Devé³ containing 10.5 liters.

Number and Condition of the Cysts.

The size of the cysts varied from pea size to the enormous dimension in the case just described. The old, dead cysts were of varying sizes, most frequently from billiard ball size to that of a large orange. Completely calcified cysts were frequently the size of a billiard ball, and so hard that they could not be cut through with a knife. When sawed through they were frequently found calcified through and through and hardly any rests visible of a parasitic membrane. In most cases only a solitary cyst was found; in some cases they were multiple. The number of cysts in the liver is an indication of the massiveness of the original infestation.

In the liver we found: 1 cyst in 37 cases, 2 cysts in 12 cases, 3 cysts in 5 cases, and 4 or more in 2 cases.

Of 56 hepatic cysts 19 (34%) were multiple. Dew⁴ found 31% at operations.

Where multiple cysts were found their condition was usually similar, although the size might vary considerably. Yet one might be found completely calcified and the other only with a chitinous membrane and containing a gelatinous brownish mass. In no case did we find an accompanying cirrhosis, as described by Devé and others.

Comment. These findings must be taken as representative for conditions as they

have been during the last 13 years in this country. They are from hospitals in Reykjavik, which receive patients from all over the country, although the majority are inhabitants of Reykjavik. But as a considerable proportion of Reykjavik's inhabitants consists of persons who have been brought up in the country, our material should reflect the present conditions as accurately as can be expected in a survey of this kind.

For comparison it may be of interest to observe the numbers of reported echinococcus cases from the time such reports are available. Figure 1 shows the enormous decline which has taken place in the number of these patients. If we apply Magnússon's method to find out the number of echinococcus cases, we see that their number is only a tenth part in 1940 of what it was in 1912, when he made his estimate, that 1 out of every 240 had an echinococcus. According to his estimate there should be 10 times fewer echinococcus cases now, or 1 in every 2400 inhabitants. As we find an echinococcus in 1 out of 20, the difference is enormous and cannot be explained by the assumption that only 1% of echinococcus carriers can be regarded as patients. In our material 10% of the parasites had been clinically manifest prior to death and certainly a very considerable proportion of dead parasites of orange size must have caused clinical symptoms formerly, which in some cases was evident from the anamnesis.

The explanation of this great discrepancy is simply that even the most experienced and sagacious investigators have not been able to estimate with anything approaching exactness the distribution of hydatid cysts in this country, as long as they had no autopsies on which to base their calculations. The population has been more heavily infested with the parasites than anybody could imagine. Probably a third or even a half of the adult population has harbored an echinococcus at the close of the last century. We can obtain an idea of the situation by observing the findings in the old people during

the first years of our autopsy work. From 1930 to 1934 we performed autopsies on 32 individuals past 60 years of age. In 11 of these 32 persons, hydatid cysts were found. These people have been about 30 years old at the close of the last century and the parasites were probably all older than that.

poverty was widespread and lack of cleanliness very common. Under these circumstances the parasite had obtained almost ideal conditions for its subsistence. Krabbe⁸ found *Tænia echinococcus* in 28% of Icelandic dogs in 1862 and showed that this infestation rate was 47 times higher than in Denmark. Not only were the dogs

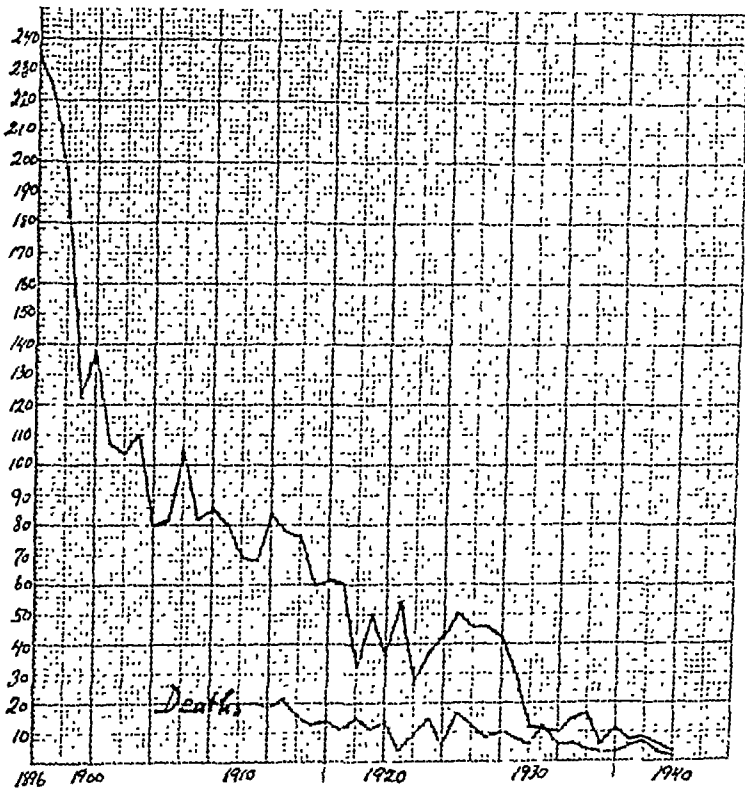


FIG. 1.—Number of reported echinococcus patients in Iceland from 1896–1940 and reported deaths from echinococcosis since 1911.

Discussion. It is evident that echinococcosis has been more widespread here than even the highest estimates supposed. This is not surprising, considering the living conditions of the people, who lived in close communion with dogs and were completely ignorant about the nature and contagiousness of the disease. And this state of ignorance continued through centuries, when the entire population lived under primitive conditions as sheep breeders, when everybody was exposed to contact with dogs and every dog had access to the entrails of sheep. Extreme

highly parasitized, but their number was enormous (15 to 20,000) at a time when the population was only 70,000.

In a medical report 1858 Finsen mentions that an old ewe or a cow is very rarely slaughtered without finding an echinococcus cyst. Probably the condition of the human population would have been found similar, if proper investigations had been made.

There can be little doubt that the young generation now growing up in this country is practically free from echinococcosis, as is borne out by our autopsy records. The

parasites are only harbored by the old people and it is to be expected that the present young generation will show a very low echinococcus rate when its time comes to take the place of the old generation.

The *causes* of this decrease in parasitism are several. First and foremost is the education of the population. The causal relationship between hydatid cysts in people and sheep on the one side and the *tænia* in the dogs on the other side has been explained to the entire population and even in the children's schools.

Slaughtering of sheep is done under such conditions that dogs have no access to the entrails of sheep.

Every dog in the country is given an anthelmintic once a year. The effect of this measure is seen in the disappearance of all kinds of helminthic cysts in sheep, also the *Cœnurus cerebralis*, which used to be seen more or less in every flock of sheep in the last century.

The further cause of the decrease of parasitism is the change which has taken place in the sheep breeding industry.

Formerly sheep were grown until they were 3 to 4 years old before they were killed. By that time their hydatid cysts were old enough to have developed scolices. Now the lambs are killed when 5 months old, but comparatively few old sheep are slaughtered.

One more reason has been added during the last 3 decades: The rural population has moved to the towns and villages, and the fact that one-third of the entire population lives in Reykjavik, greatly diminishes the danger of exposure for that part of the population.

Summary. In a total of 1231 autopsies 60 hydatid cysts were found. The young generation is practically free from this disease which is found with increasing frequency in the higher age groups. The infestation rate among the oldest age groups is a remnant of the old conditions, when the population had been heavily parasitized.

The causes for the improvement in these matters are discussed.

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TRAUMATIC STREPTOCOCCIC MENINGITIS

REPORT OF A CASE WITH RECOVERY AND REVIEW OF LITERATURE*

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SINCE the advent of sulfonamide therapy, and more recently penicillin, reports of successfully treated streptococcic meningitis have become commonplace. Of 102 cases reported from 1935 to 1939, 81 (79.4%) had recovered;¹² whereas prior to present-day chemotherapy the estimated mortality was 97%.⁴ Such cases were nearly all of otogenic origin. So-called post-traumatic meningitis, on the other hand, and particularly when secondary to a streptococcus, is exceedingly rare. Thus, careful perusal of the literature^{1,2,3,5,6,8,9} reveals only 8 cases cured by

traumatic origin the following case was considered worthy of adding to the literature. The long incubation period as well as the unusual strain of organism are of interest and may have been factors influencing the favorable outcome of the patient's illness.

Case Report. G. L. R., 31 year old garage attendant, entered the Veterans Hospital, San Francisco, Feb. 15, 1945 (his second admission) with a tentative diagnosis of meningitis. He was first admitted into the hospital on January 15 for treatment of a nasal laceration, 10 days after a fall against

TABLE 1.—CASES OF TRAUMATIC STREPTOCOCCIC MENINGITIS SUCCESSFULLY TREATED FROM 1901 TO 1935 (PRE-SULFONAMIDE ERA)

Author	Cases	Age	Injury	Organism	Treatment
Barth (1914)	1	19	Back trauma	Streptococcus	Laminectomy
McCarthy <i>et al.</i> (1917)	1	25	Head trauma	Streptococcus	Spinal drainage and anti-streptococcic serum
Dandy (1924)	1	6	Head trauma	Streptococcus	Surgical drainage
Canuyt <i>et al.</i> (1933)	1	11	Trauma to eyelid	Streptococcus	Spinal drainage and anti-streptococcic serum
Zeligs (1935)	1	6	Basal fracture	Streptococcus	Spinal drainage and anti-streptococcic serum

means of sulfonamides (Table 2). In contrast only 5 cases were reported cured during the pre-sulfonamide era of 1901-1935 (Table 1).

The majority of cases of traumatic meningitis encountered in the literature were found to be due to a pneumococcus. Trauma, as a rule, occurred to the head or face with resultant skull fracture in practically all cases. Those in whom a cerebrospinal leak was demonstrated were especially vulnerable to meningeal infection.

Because of the rarity of successfully treated streptococcic meningitis of trau-

a screen door. The laceration had been sutured originally at one of the city emergency hospitals, and had apparently healed by primary intention. The day before his first entry he reinjured his nose, reopening the old laceration. Roentgen ray revealed a comminuted fracture of the nasal bone and it was concluded that the fracture was compounded. The distal fragment was elevated by splints and a secondary closure of the soft tissues was carried out. The wound healed normally and he was discharged from the hospital on February 3. He returned to work and felt well until the day prior to his present (second) admission. At this time he became ill with headache, malaise and low

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back pain. Within the course of the next several hours he became acutely prostrated with chills, fever, nausea, vomiting and stiff neck.

The past history disclosed the following facts: disability discharge from the U. S. Army in 1943 because of duodenal ulcer; malaria in 1931 with relapse in 1932; and renal colic in 1932 with passage of a calculus.

disclosed no organisms. White blood cell count was 8800 (85% neutrophils).

Treatment. The patient was immediately given 5 gm. of sodium sulfadiazine by the intravenous route, and 20,000 units of penicillin intramuscularly. Several hours later the vomiting subsided and he was able to take $1\frac{1}{2}$ gm. of sulfadiazine orally every 4 hours. Penicillin was continued every

TABLE 2.—CASES OF TRAUMATIC STREPTOCOCCIC MENINGITIS SUCCESSFULLY TREATED BY MEANS OF CHEMOTHERAPY

Author	Cases	Age	Injury	Incubation, days	Organism	Treatment
Frazer (1937)	1	17	Skull fracture	7	Hem. strep.	Prontosil and spinal taps
Davel (1937)	1	16	Skull fracture, cerebro-spinal otorrhea	10	Hem. strep.	Prontosil and spinal taps
Ricard <i>et al.</i> (1938)	1	?	Basal skull fracture, cerebrospinal rhinorrhea	4	Hem. strep.	Sulfanilamide and polyvalent serum
Narat (1939)	1	52	Skull fracture (through frontal sinus) and nasal fracture	3	Hem. strep.	Sulfanilamide
Gurdjian (1941)	2	14	Basal fracture, cerebro-spinal otorrhea	5	Hem. strep.	Sulfanilamide
		29	Basal fracture, aural bleeding	5	Hem. strep.	Sulfanilamide
Boatman (1941)	1	25	Spinal injury by shrapnel	21	Hem. strep.	Laminectomy and prontosil
Riley and Waugh (1943)	1	33	Skull fracture (involving frontal sinus)	2	Non-hem. strep.	Sulfapyridine
Berk (1945)	1	31	Nasal fracture, compound	32	Non-hem. strep.	Sulfadiazine and penicillin

Physical examination revealed an acutely ill, restless, rational white man, who complained of severe headache and low back pain. The neck was rigid and a bilateral Kernig sign was present. Otoscopic examination was negative. Percussion over the entire spine disclosed tenderness. The skin was hot and there was evidence of dehydration. The temperature was 102° F., pulse rate 120, and respiratory rate 24. Blood pressure 110/80. A horseshoe-shaped recently healed scar about 4 inches in length extended across the bridge of the nose. At its mid-point there was some soft tissue thickening but no signs of active inflammation.

A lumbar puncture revealed turbid fluid under 180 mm. initial pressure with a cell count of 8600, nearly all of which were polymorphonuclears. Spinal sugar content was less than 10 mg. per 100 cc., and the protein was 333 mg. per 100 cc. Immediate smears

3 hours. Approximately 24 hours after admission 10,000 units of penicillin in 10 cc. of saline was injected intrathecally after a like quantity of spinal fluid was removed. A short while later his back pain became aggravated, with radiation down the lower extremities. Morphine was required for relief. After 48 hours, culture of the spinal fluid showed a non-hemolytic streptococcus, which later proved to be micro-aërophilic. Blood culture was negative.

In view of the favorable clinical response at the end of this time and because of the untoward reaction to intrathecal penicillin the day previously, further medication by this route was not deemed advisable. At the end of the 3rd day the patient's temperature had reached normal and his neck rigidity had disappeared. The spinal fluid on the 5th day was clear for the first time with a cell count of only 120, most of which were polymorphonuclears. Dextrose value

was 26 mg. %. Smear and culture of spinal fluid were negative. On the 8th day the spinal cell count was 56, only 24 being polymorphonuclears. The sugar content had risen to 100 mg. %, and the protein value was reported as 48 mg. %. Culture was negative.

Penicillin was discontinued after 5 days, a total of 520,000 units having been administered intramuscularly and 10,000 units intrathecally. Sulfadiazine was continued for 10 days, the patient having received 56 gm.

The patient was discharged as recovered without complication or sequela on March 6, after less than 3 weeks hospitalization. His recovery was attributed primarily to sulfadiazine, owing to the fact that clinical and cultural response had been manifest within the 1st 24 hours of his illness, which was prior to the injection of intrathecal penicillin. The effect derived from the intramuscular penicillin was considered of minimal degree since it has been shown⁷ that when administered in this manner it does not pass the meningeovascular barrier in significant quantities. On the other hand, although only 1 injection was given intraspinally, its effect probably played no small part in the successful outcome. This conclusion is borne out by Rosenberg and Arling's¹⁰ report of 40 cases of meningitis treated by penicillin in which the majority of patients required only 1 or 2 intrathecal injections.

Comment. The portal of entry of the offending organism in the above case was probably through a defect in the cribiform plate. Schroeder¹¹ mentions 2 additional sites where a defect is apt to occur in nasal injuries, with resulting communication between the nasal cavity and cerebrospinal system, namely, in the posterior wall of the frontal sinus with a tear in the dura, and defects in the walls of the

sphenoid sinus. Trauma to the nose or face may lead to any one of these fistulous communications and thus invite infection of the meninges by an organism which inhabits the nasal passages at the time.

Of the 8 tabulated cases, 7 suffered a head injury with skull fracture in all but 1. Two patients had in addition fractures involving the frontal sinuses, which may have been the portal of entry. Three cases with basal fractures presented cerebrospinal otorrhea, whereas a fourth suffered bleeding from the ear. Thus, one may conclude otitic origin of infection in the latter 4 cases. Boatman's patient was the only one whose meningitis originated in an injury below the head. The author's case, on the other hand, is the only one secondary to an uncomplicated nasal injury.

Summary. 1. A case of traumatic streptococcic meningitis, secondary to a compound nasal fracture, and successfully treated with sulfadiazine and penicillin is hereby presented.

2. Only 8 cases of traumatic streptococcic meningitis cured by chemotherapy are found in the literature, to which a ninth is added by the author.

3. The author's case is the first reported due to a non-hemolytic (micro-aërophilic) streptococcus and cured by chemotherapy.

4. Prophylactic sulfonamide and penicillin should be given in all cases of traumatic cerebrospinal fluid leaks.

5. Traumatic meningitis due to pneumococcus occurs more frequently than meningitis due to streptococcus. This might be explained by the predominance of the former organisms in the nasal and aural passages.

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PNEUMONITIS OCCURRING IN RHEUMATIC FEVER

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PNEUMONITIS occurring in the course of rheumatic fever is one of the more prominent of the protean manifestations of rheumatic fever. American and English writers have spoken of the rheumatic pneumonias and described in part the clinical picture. Fuller³ and Garrod⁴ described rheumatic pleurisy as part of the rheumatic fever entity. Fuller has written of the frequency of the occurrence of pneumonias in acute rheumatic fever. Garrod mentioned the transient character of the physical signs. In recent years Thayer⁵ and Swift⁷ described the clinical picture and noted the frequency of rheumatic pneumonitis and pleurisy. In 1928,

matic fever and occurring in such a case." In 1932, Gouley and Eiman¹ again published their findings in the lungs in 9 cases of acute rheumatic fever. They were similar to the description quoted above.

Definition. Pneumonitis is a manifestation of rheumatic fever characterized by an inflammatory process of the lung and pleura, with an insidious onset, migrating consolidation, and frequent pleurisy with or without effusion.

Source of Material. In a group of 1046 rheumatic fever patients observed in the rheumatic fever unit at the U. S. Naval Hospital, pneumonitis was found in 119 cases.

TABLE 1.—OCCURRENCE RATE OF PNEUMONITIS

Type	No. patients with rheumatic fever	No patients developed pneumonitis	%
Acute fulminating	52	28	53.1
Polycyclic	281	77	27.4
Monocyclic, subacute	667	14	2.0
Subclinical	46	None	0.0
Total	1046	119	11.3

Paul⁶ presented a very complete historical review and outlined the physical findings in detail. In 1928, Gouley and Eiman² described the gross and histologic pathologic findings, and based their opinion of the specific nature of rheumatic pneumonitis upon "the histologic study of the lungs showed an acute interstitial inflammatory process, apparently hematogenous in origin, with hyperemia, edema and a characteristic cellular exudate, perivascular, consisting of large endothelial cells, giant cells, plasmocytes, lymphocytes and a few neutrophils, the distribution of this exudate and its general appearance being identical with the cardiac lesions in rheu-

Occurrence. The occurrence rate in this group of patients was 11.3%. Table 1 shows that the most frequent occurrence was in the acute and polycyclic types of the disease. No demonstrable pneumonitis was found in the subclinical types.

Pathologic Anatomy. The gross and histologic pathology are discussed in detail in recent papers by Huntington and the author.⁵ In short, gross examination shows areas of mottling which are characteristic of infarction. The cut surface is smooth and, although hyperemic in appearance, is relatively bloodless. The mottled areas may be found in all lobes of the lungs. The histologic picture is

that of an anaphylactic angiitis involving the larger vessels as well as the capillaries. There is endothelial proliferation, hemorrhage, necrosis and hyalinization. Perivascular infiltration of plasmocytes, giant cells, lymphocytes, myocytes with owl-eyed nuclei and relatively few neutrophils

there is little residual evidence of the pleuritis. An effusion is found relatively infrequently. In this series of cases effusion occurred in 10.9% of the cases. Effusions tend to be temporary. The fluid is sterile and straw to sanguinous in color, depending upon the number of red



FIG. 1.—Lung. Pneumonia with alveolar exudate made up mostly of fibrin, large monocytes and lymphocytes. Note the amorphous "hyaline" character of some of the acellular material. The blood vessel marked by the arrow is involved, apparently, in the inflammatory process.

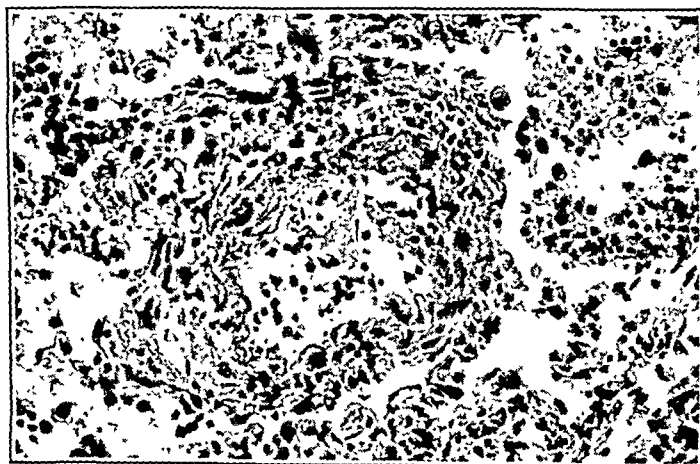


FIG. 2.—Inflammatory reaction in small artery in lung. (Autopsy 3), H. & E.

Figures 1 and 2 were photographed by Dr. R. W. Cragg at the U. S. Naval Medical School. The remaining figures are the work of Mr. Edward N. Hamilton at the College of Medical Evangelists, Los Angeles.

is characteristic. Aschoff bodies may be found freely in various stages of development and maturity. The alveoli are filled with collagen, fibrin and blood cells. The pleura may show a fibrinous exudate on both the parietal and visceral membranes. Organization and formation of fibrous adhesions may occur, but more frequently

cells. At times large numbers of eosinophils are found in the white cell count. The effusion may absorb very rapidly with very little residual evidence of the pleurisy.

Types of Pneumonitis. There are 3 clinical types of pneumonitis recognizable: (1) primary acute pneumonitis, (2) secon-

dary acute pneumonitis, and (3) subclinical pneumonitis.

1. *Primary acute pneumonitis* may be and is at times the *presenting manifestation* of rheumatic fever. The history of an upper respiratory infection several weeks previously, a period of latency, the development of shortness of breath on exertion, cough, occasional blood spitting, fever of moderate to high degree, tachycardia of greater degree than that usually found with the height of the temperature, cyanosis and restlessness are the symptoms and signs by which this manifestation may be suspected. On physical examination an area of dullness may be found in 1 or more lobes, accompanied by a suppression of the breath sounds and a few fine, crackling râles. This area may develop into frank consolidation with increased dullness and tubular breathing. More frequently, however, the original area clears in 2 to 3 days and a new area develops. After 2 or 3 days other manifestations of rheumatic fever appear, such as migrating polyarthritis, carditis and a sustained relatively high fever. The diagnosis is confirmed by finding an area of cloudiness in 1 or more lobes of the lungs from Roentgen ray study. There is a *leukocytosis of moderate degree*, without a marked shift to the left in the Schilling index. The sputum study usually shows no pathogenic organisms and is relatively free of leukocytes. The sedimentation rate is usually high, and at times there is electrocardiographic evidence of myocardial change. The course of the primary acute type of pneumonitis is severe and prolonged. The pneumonitis may become so widespread as to cause a very greatly decreased oxygen carbon dioxide exchange, with marked cyanosis and air hunger. At the same time the *pulmonary arterial tension* rises to such a level that there may be primary right-sided heart failure. The heart which is the seat of a severe carditis is unable to meet the increased work load thrown on the right ventricle, and primary right ventricular failure may ensue. It is

the opinion of the clinicians working in this group that where there is primary right-sided heart failure in rheumatic fever, pneumonitis is one of the initiating causes. The pneumonitis subsides only as the other manifestations of the rheumatic fever subside. The physical signs of the pneumonitis clear up long before the Roentgen ray findings become negative.

Table 1 shows that 53.1% of the acute fulminating type of rheumatic fever had rheumatic pneumonitis and in approximately one-half of these cases the initial symptoms were referable to the lungs. Most of the patients were treated for a primary atypical pneumonia until other manifestations of rheumatic fever appeared, such as polyarthritis and carditis.

2. *Secondary acute pneumonitis* occurs during the course of established rheumatic fever. From Table 1 it is noted that secondary acute pneumonitis occurs most frequently in the polycyclic type of rheumatic fever. The actual occurrence rate was 27.4%. The onset may begin late in the first cycle, but more frequently it is one of the presenting manifestations of the second or third cycle of the polycyclic type.

The symptoms are those of shortness of breath, cough, pain and restlessness. Cough may be primary and severe, or mild to absent. Pain is usually late in its appearance as a symptom, but is severe and prolonged unless an effusion develops. Cyanosis is present only if involvement of the lungs is widespread. Restlessness is an early and persistent symptom.

The first signs to appear are fever and tachycardia. Dullness over localized areas of the lungs appears early and may shift from 1 lobe to another or within the same lobe. The dullness may increase to flatness with the development of massive involvement of a lobe or with the rapid pouring out of a pleural effusion. Early the breath sounds are diminished over the area of dullness. As the area of involvement increases, tubular breathing may develop but this is relatively infre-

quent. Râles at first are small and finely crackling. Later the râles increase in number and become more moist in nature. The signs may clear fairly rapidly, but they may, however, persist as long as other manifestations are present. The Roentgen ray study confirms the diagnosis by revealing an area of cloudiness at the site of the physical findings. The fever, tachycardia, the leukocytosis and blood sedimentation rate are elevated usually in proportion to the severity of the rheumatic process.

Roentgen Ray Findings. The characteristic findings in the Roentgen ray studies in rheumatic pneumonitis and pleuritis are the rapid onset and clearance of densities in one area to suddenly reappear in another area in the lung fields, and the equal rapidity with which a pleural effusion may appear and then as quickly disappear, and the close association of the pneumonic densities to the bronchovascular markings spreading into the adjacent parenchyma. At the onset the area of cloudiness is similar to that

TABLE 2.—PNEUMONITIS—LOBES INVOLVED

Location	No of cases
Central hilar area	16
Middle lobe	11
Upper lobes	18
Basal lobes	26
Multiple lobes	48
Total	119

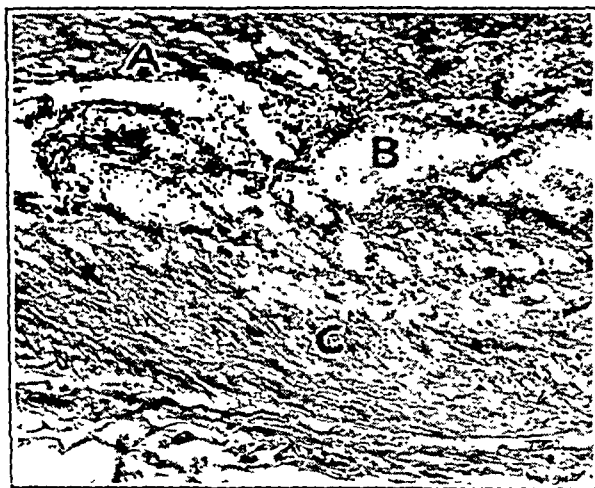


FIG. 3.—Thrombus (A) with beginning recanalization (B) in medium-sized pulmonary artery, arterial wall is (C). (Autopsy 6.) Masson Trichrome, $\times 125$.

3. *Subclinical pneumonitis* is found accidentally. There are no symptoms and few, if any, physical signs. The subclinical pneumonitis is suspected when a rheumatic fever patient is more ill than the clinical findings would indicate. The diagnosis is based entirely on the Roentgen ray finding of an area of cloudiness either in the hilar area or in isolated fields of the lungs (see Table 1). Subclinical pneumonitis of rheumatic origin occurred in 2% of the subacute monocyclic cases.

seen in primary atypical pneumonia and that seen in sulfonilamide sensitivity. There is localized patchy density without much shift of the mediastinum. At times the area of cloudiness may be triangular and near the periphery, resembling an infarction; and again it may be circular and central. Table 2 shows the distribution of pneumonitis according to lobes involved. Multiple and upper lobe involvement occurred more frequently in the very ill patients.

Pleurisy was not found as frequently as

the early writers indicated. Most of the cases of pleurisy were confirmed by Roentgen ray findings. Effusion accompanying pleuritis is not infrequent, occurring in 18.8% of cases of demonstrable pleurisy. The percentage of occurrence of pleuritis



FIG. 4.—Small pulmonary vessel (Autopsy 6), showing edema and cellular accumulation beneath inner layer. Masson Trichrome, $\times 500$.

would be much higher if pain is taken as the diagnostic criterion. To make the diagnosis of pleurisy, pain, pleural friction rub and positive Roentgen ray findings of thickened pleura, peripheral pneumonitis and/or effusion were required.

fever appear. The concomitant manifestations which are most helpful in establishing the diagnosis of rheumatic pneumonitis are carditis, migrating polyarthritides, purpura, erythema marginatum, epistaxis and subcutaneous nodules.

Bacterial pneumonia is differentiated by the sudden onset with chill, high fever, characteristic sputum, Roentgen ray findings of lobar involvement, and the laboratory determination of the causal organism.

Tuberculous pneumonia is differentiated by the history of exposure, by the course of the pneumonia and the absence of the protean manifestation of an active rheumatic state.

Pleurisy with effusion cannot be differentiated except by guinea-pig inoculation. The development of carditis, migrating polyarthritides, erythema marginatum and subcutaneous nodules aid in the differential diagnosis pointing to rheumatic fever rather than to tuberculosis.



FIG. 5.—Fibrinous and cellular exudate in lung. Rupture of capillary at arrow. (Autopsy 6.) Masson Trichrome, $\times 125$.

FIG. 6.—Lung. Showing border of infarcted area (Case 6). $\times 80$.

Differential Diagnosis. The differential diagnosis is not easy. The primary acute pneumonitis cannot be distinguished from primary atypical pneumonia either clinically, by the sputum studies, by Roentgen ray, or by blood studies. The pneumonitis of rheumatic fever is recognized when the other manifestations of rheumatic

The differentiation of rheumatic pneumonitis from congestive heart failure is based upon the following facts. In congestive heart failure air hunger and increased venous pressure are more marked. In congestive failure there is an enlarged liver which is tender and pulsates. Ascites and dependent edema soon follow. A tri-

cuspid systolic murmur is frequently heard. Since pneumonitis frequently precedes congestive failure, careful observation and timing of events is necessary to state where the pneumonitis is complicated by congestive failure.

with a moderate increase in the granulocytes in all types of lung involvement except the primary atypical pneumonia. The antistreptolysin titer is definitely elevated in rheumatic fever, with or without a pneumonitis, and serves to

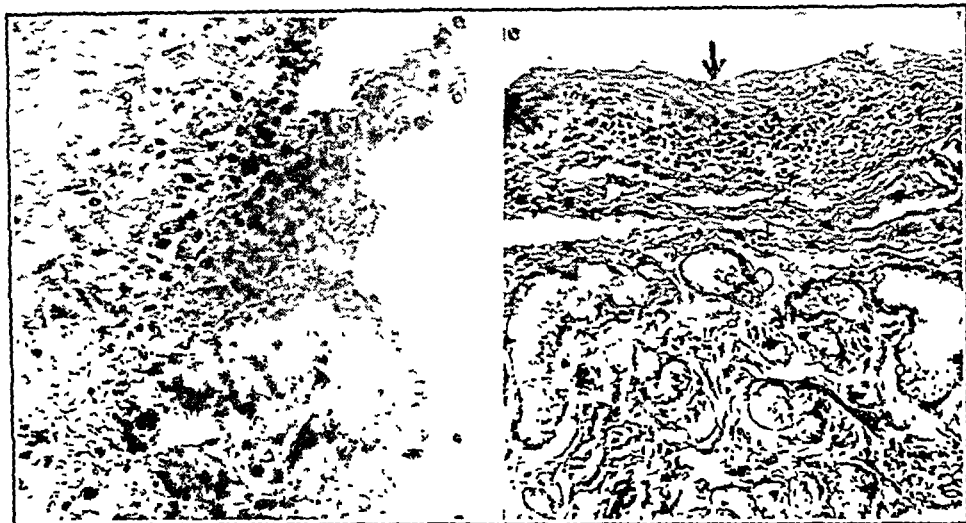


FIG. 7.—Vegetation in large branch of pulmonary artery (Case 8). Masson Trichrome, $\times 320$.

FIG. 8.—Pleural surface (arrow) with group of mononuclear cells. Subpleural group of dilated vessels (Case 8). Masson Trichrome, $\times 160$.

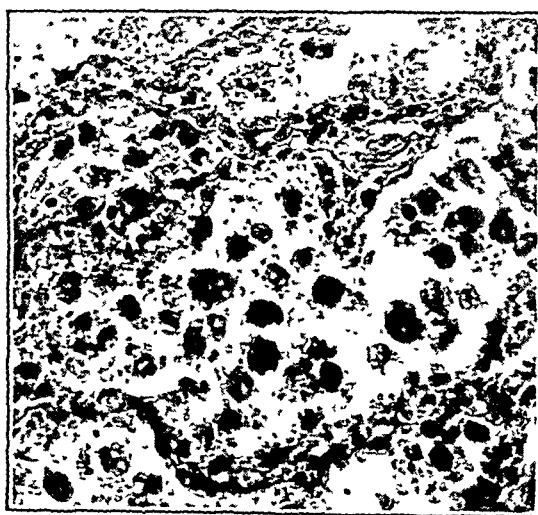


FIG. 9.—Alveolus, showing cellular detail (Case 8). Masson Trichrome, $\times 320$.

The laboratory aids are of little help in the differential diagnosis except in lobar pneumonia. The blood sedimentation rate is elevated approximately to the same level in primary atypical pneumonia, in tuberculous pneumonia and in rheumatic pneumonitis. The leukocyte count is elevated as high as 20,000 to 25,000 cells,

differentiate rheumatic pneumonitis from the primary atypical pneumonia, bacterial pneumonia and from tuberculosis.

Discussion and Conclusions. 1. Pneumonitis is one of the prominent manifestations of rheumatic fever activity.

2. Pneumonitis is a definite pathologic entity only when considered as one of the

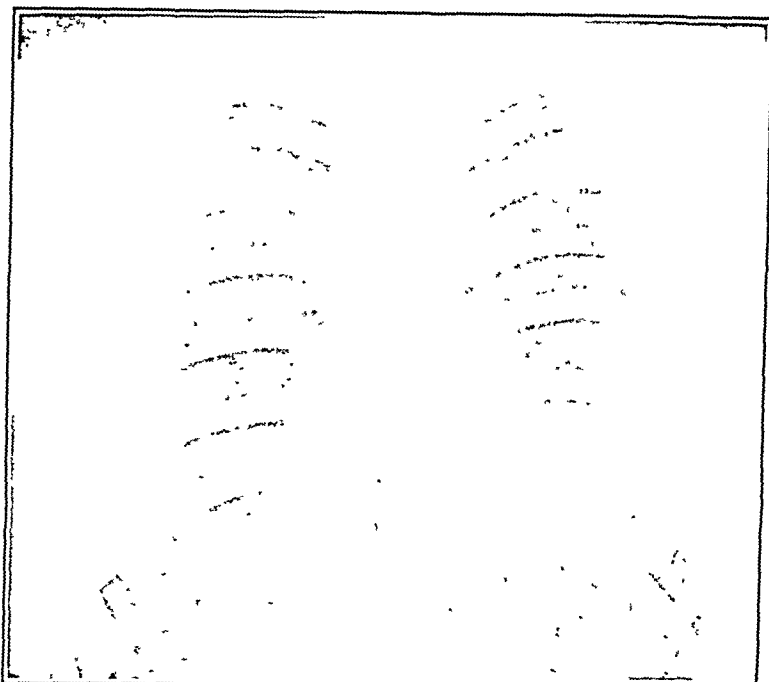


FIG. 10.—Case 1, No. 15328. Rheumatic pneumonitis. Radiograph of the chest reveals a diffuse patch of increased density at the level of the third left anterior interspace.

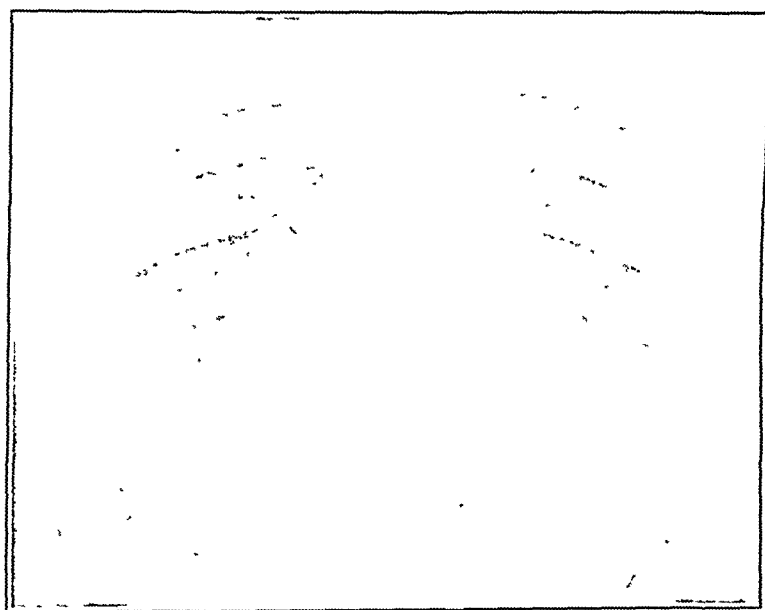


FIG. 11.—Case 2, No. S443. Rheumatic pneumonitis with associated pleural changes. Radiograph of the chest reveals a patch of increased density at the level of the fourth and fifth right anterior interspaces. There is associated haziness about this area consistent with pleural changes.

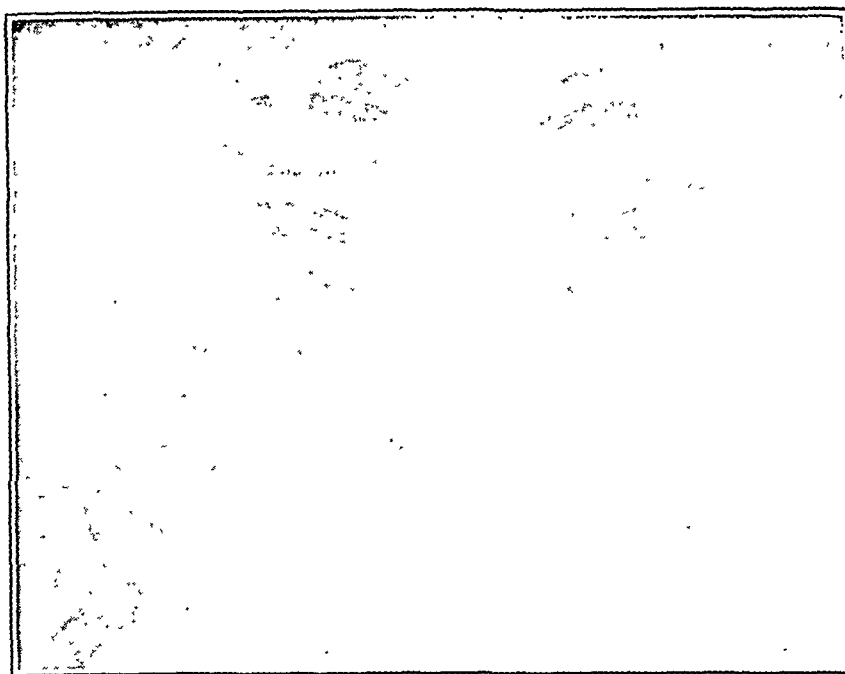


FIG. 12.—Case 3 (radiograph No. 1), No. 18849. Rheumatic pneumonitis. Radiograph of the chest reveals multiple patches of increased densities throughout both lung fields.

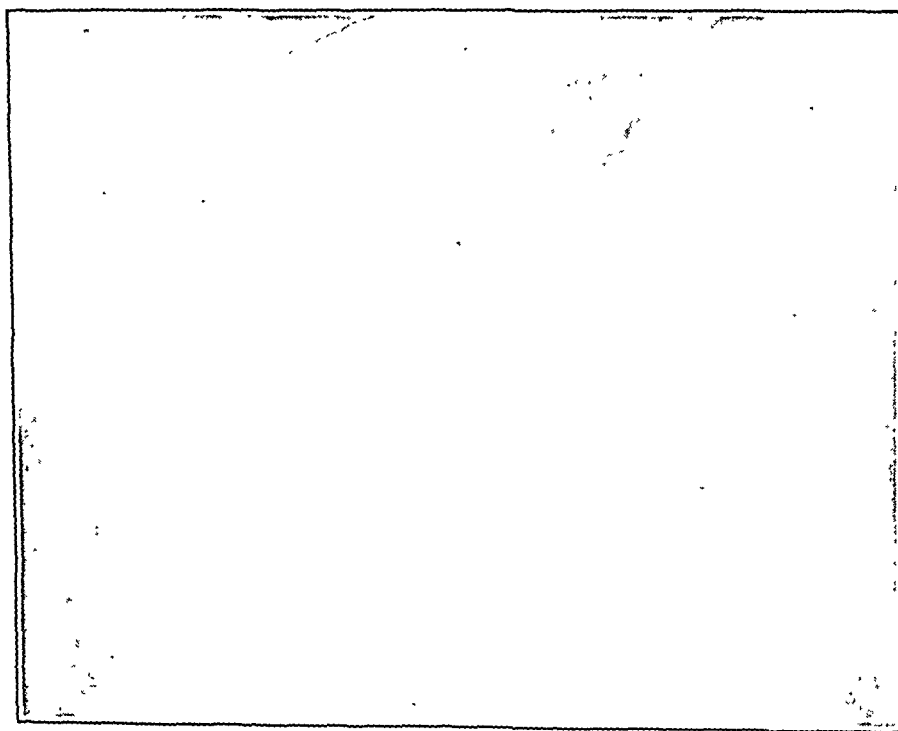


FIG. 13.—Case 3 (radiograph No. 2), No. 18849. Rheumatic pneumonitis and terminal pulmonary congestion. Radiograph of the chest 3 days following previous radiograph now reveals diffuse homogeneous densities throughout the inner and middle zones of both lung fields.

manifestations in the widespread angitis of rheumatic fever.

3. Pneumonitis occurs in approximately 11% of rheumatic fever cases. It is seen in 53.1% of the acute fulminating type; in 27.4% of the polycyclic type, and in 2% of the mild monocyclic type of rheumatic fever.

4. The pneumonitis is of 3 types, according to its time of appearance in the rheumatic fever state: primary acute, secondary acute, and the subclinical.

5. The diagnosis of pneumonitis of rheumatic origin is based entirely on the exclusion of the other types of pneumonia and the concomitant development of other manifestations of acute rheumatic fever.

6. Roentgen ray findings in pneumonitis of the rheumatic type are not specific; but the rapid shift of areas of density, the rapid development of an effusion and the close adherence of the density to the bronchovascular markings are helpful findings.

7. There is no specific laboratory test. The laboratory aids are of little help in establishing the diagnosis.

8. The importance of pneumonitis of rheumatic fever origin as one of the serious manifestations of rheumatic fever activity cannot be overemphasized.

Summary. A study of 119 cases of pneumonitis, with histologic and Roentgen ray illustrations is presented.

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SELECTION OF PATIENTS WITH ARTERIAL HYPERTENSION FOR TREATMENT BY REPEATED INJECTIONS OF PITRESSIN

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In 1941 Griffith, Corbit, Rutherford and Lindauer¹ described a group of patients with high blood pressure whose sera contained a substance capable of suppressing water diuresis in rats. It was suggested that this antidiuretic substance was, in fact, the antidiuretic hormone derived from the posterior lobe of the pituitary, and that the hypertension in these patients was due to increased activity of the posterior lobe of the pituitary. Subsequently, Pendergrass, Hodes and Griffith⁴ and later Pendergrass, Griffith, Padis and Barden⁵ described the result of treatment of such cases by pituitary irradiation. About half the patients so treated showed a drop in blood pressure, frequently to the normal range, along with clinical improvement, both occurring 1 to 3 months after the irradiation and associated with disappearance of the antidiuretic substance from the blood stream.

In 1940 Robinson and Farr⁶ described a treatment for patients with edema, by repeated injections of aqueous pitressin. They found that such injections, if given daily, would increase the antidiuretic titer of the urine for a few days, but subsequently the urine would become actually diuretic as compared with its pre-injection level. They suggested that this effect was due to the appearance in the body of an antagonist to pitressin, and showed that, at the time when it appeared, edema was usually cleared. It is possible that the explanation may be the development of an antihormone, but this has not yet been established.

It seemed probable that repeated injections of pitressin might have an effect such as had previously been observed following irradiation. In the beginning small doses of the aqueous preparation were used varying from 0.2 to 1 cc. (Pitressin, Park Davis, each cc. containing 20 pressor units). Patients were selected on the same basis as for pituitary irradiation, as follows: (1) Bio-assay for antidiuretic hormone in serum must be positive; (2) bio-assay for gonadotropic hormone in serum must be negative at the level of 330 mouse units per 100 cc. (the method used was that described by Rakoff⁷); (3) renal function must be normal as tested by the plasma creatinine method described by Steinitz and Turkand.⁸ In addition, patients were selected who were less than 60 years of age who had suffered no previous vascular accident. They were also studied from the standpoint of capillary reactivity by the method described by Griffith, Roberts and Corbit,² and cutaneous lymphatic flow by the patent blue method of McMaster,³ although neither of these tests was used in actual selection. Subsequently pitressin tannate in oil became available (Park Davis, 1 cc. = 5 pressor units) and this was substituted for the aqueous preparation.

Material and Method of Study. In all, 63 patients with arterial hypertension were studied, treated, and followed for a period varying from 3 to 26 months, averaging 8 months; 33 of these were males, and 30 females. Ages ranged from the 2nd to the 6th decade, with only 5 subjects below 30.

* Atwater Kent Fellow in Medicine.

TABLE 1.—EFFECT OF INJECTIONS OF PITRESSIN UPON BLOOD PRESSURE

		A. Systolic blood pressure before injections*				
		200-250	170-200	150-170	Below 150	
Systolic blood pressure after injections	200-250	5				
	170-200	4	16			
	150-170	(3)	11	9		
	Below 150	(1)	(4)	(10)		
		B. Diastolic blood pressure before injections				
		Over 140	120-140	110-120	100-110	Below 100
Diastolic blood pressure after injections	Over 140	1				
	120-140		7			
	110-120		4	9		
	100-110		(3)	6	5	
	Below 100		(3)	(7)	(7)	11

Numbers refer to number of patients. Those in parentheses are thought to show good results and those in bold face fair results.

TABLE 2.—CORRELATION BETWEEN CHANGE IN SYSTOLIC AND IN DIASTOLIC BLOOD PRESSURE FOLLOWING INJECTIONS OF PITRESSIN*

		Systolic blood pressure change		
		Good	Fair	No change
Diastolic blood pressure change	Good	11	8	1
	Fair	1	6	2
	No change			23

* The 11 patients whose initial diastolic blood pressure was below 100 are omitted from this correlation. Of these the systolic change was good in 6 cases and no change in 5.

TABLE 3.—CORRELATION BETWEEN CHANGE IN SYSTOLIC BLOOD PRESSURE AND CLINICAL IMPROVEMENT

		Systolic blood pressure change		
		Good	Fair	No change
Clinically	Good	10	6	
	Probably good	8	5	7
	No change		4	23

TABLE 4.—FACTORS INFLUENCING THE RESPONSE TO INJECTIONS OF PITRESSIN TANNATE IN OIL IN HYPERTENSION

Group:	1	2	3	4	5	6	7
Clinical and blood pressure	good	Clinical good, blood pressure fair	Clinical probably good, blood pressure good	Clinical probably good, blood pressure fair	Clinical probably good, blood pressure no change	Clinical no change, blood pressure fair	Clinical and blood pressure no change
After injections, bio-assay antidiuretic hormone:							
Negative	10	5	8	5	5	3	11
Positive	..	1	2	1	12
Pitressin:							
(a) Aqueous	1	..	4	.	1	..	7
(b) In oil:							
Less than 6 injections			..	5	5	4	9
More than 6 injections	9	6	4				7
Before treatment cutaneous lymphatic flow:							
Normal	6	3	5	3	5	1	5
Increased	3	3	2	2	2	3	10
Before treatment cutaneous capillary reactions:							
Normal	10	5	4	3	6	4	15
Abnormal	..	1	1	2	1	..	3

Treatment may be divided into 3 general types: (1) 13 patients were given injections of aqueous pitressin in the dose previously stated, daily, for 6 injections or more; (2) 10 persons were given intramuscular injections of pitressin tannate in oil, each 1 cc., at weekly or monthly intervals, the total number of injections being less than 6; (3) 40 persons were treated similarly, but received 6 or more injections. The usual procedure was to begin with 3 injections at weekly intervals, and follow by 3 or more injections at monthly intervals, until the bio-assay for antidiuretic hormone became negative. In the beginning and for the first 3 to 6 months, bio-assays were repeated at monthly intervals; thereafter, as far as possible, every 3 months.

Results. Table 1 shows the effect upon systolic and diastolic blood pressure. There was a significant reduction in pressure in about half the subjects treated, and this at times was marked. Correlation between change in systolic and in diastolic pressure (Table 2) is good. Moreover, in Table 3, there is a good correlation between clinical result and change in systolic blood pressure. As seen in Table 3, there are 9 possible combinations of change in blood pressure along with clinical change: actually only 7 of these occurred, and these comprise the 7 groups shown in Table 4, where the best results are at the left (Group 1, etc.) and the worst at the right. However, it should be noticed that Group 7 is the only one where we are certain no benefit has been achieved. In the future, one would prefer either not to treat the patients that fall in this group, or else treat them differently. Further study of Table 4 shows that the majority of cases in Group 7 (12 out of 23) failed to show a change in bio-assay for antidiuretic hormone following therapy. It is also apparent that results were better in those patients who received 6 or more injections of the pitressin tannate in oil than in those receiving fewer injections, or in those given the injections of aqueous pitressin. Results were not significantly influenced by the state of the cutaneous capillaries or the rapidity of the cutaneous lymphatic flow.

Immediate reactions to the injections of pitressin tannate in oil did not occur, although 2 patients developed a mild transient urticaria a few hours after an injection. Immediate reactions did occur following

injections of aqueous pitressin, even in small dosage, consisting of blanching of the face, a sense of coldness and a vague sensation of being ill, and, frequently, increased peristalsis followed by 1 or more stools. The use of the aqueous preparation has been discontinued and is, in our opinion, not now justified.

Case Report. A housewife, age 47, presented herself on 2-25-44 because high blood pressure had been discovered 3 months previously at the time of a surgical operation for repair of a broken jaw. Readings had been obtained frequently during this period, and ranged from 170/110 to 230/140, more often at the higher than at the lower limit of the range. Patient complained of marked dizziness as her only symptom.

At the time of her initial study, blood pressure was 200/120, bio-assay for antidiuretic hormone was positive, bio-assay for gonadotropic hormone was negative at 330 mouse units, plasma creatinine was normal, cutaneous capillary reactions were normal as was cutaneous lymphatic flow. Pitressin tannate in oil, 1 cc., was injected intramuscularly on 3-28-44, 4-5-44, 4-12-44, 5-5-44, 6-6-44, 7-10-44, and again on 11-11-44. Blood pressure was measured on the following dates, after beginning therapy: 4-5-44 = 166/108; 4-12-44 = 144/104; 5-5-44 = 162/100; 6-6-44 = 150/98; 7-10-44 = 154/104; 9-9-44 = 170/120; 11-11-44 = 138/98; 1-13-45 = 110/70; 4-7-45 = 132/80. Bio-assay for antidiuretic hormone was repeated and was positive on 5-5-44, 6-6-44, 7-10-44, but was negative on 9-9-44 and remained negative thereafter on 11-11-44, 1-13-45, and 4-7-45. All dizziness had disappeared by 5-5-44. Patient moved to a distant city and was not followed further, but reports from her physician stated, as of 9-15-45, blood pressure had remained normal.

Summary. Sixty-three persons with hypertension were selected on the basis of: (1) Positive bio-assay for antidiuretic hormone in serum; (2) negative bio-assay for gonadotropic hormone in serum at the level of 330 mouse units; (3) normal renal function. They were given injections of pitressin tannate using various procedures but the one that appeared best was to give 1 cc. of pitressin tannate in oil weekly for

3 weeks and then monthly for 3 months, and thereafter continuing at monthly intervals until the bio-assay for anti-diuretic hormone became and remained negative. Considering the group as a whole, blood pressure was significantly lowered and symptoms improved in about half the cases. Reactions other than oc-

casional mild urticaria did not occur using the procedure just described, but severe reactions did occur when aqueous pitressin was used. The possible mechanism of the reaction is discussed on the basis of the work of Robinson and Farr,⁶ and it is suggested the results parallel closely those obtained by pituitary irradiation.

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REACTIONS TO INTRAVENOUSLY ADMINISTERED AMINO ACIDS (CASEIN HYDROLYSATES)

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PARENTERAL feeding of mixtures of amino acids and lower peptides has become a recognized therapeutic procedure in conditions associated with low nitrogen intake or high nitrogen loss. Most of these mixtures are now prepared by hydrolysis from casein and adjusted so as to contain in sufficient amounts all amino acids essential for maintenance of nitrogen equilibrium, growth and plasma protein production. Hydrolysates of the types now commercially available contain, of course, in addition considerable amounts of "non-

Mixtures of crystalline amino acids, particularly those not containing glycine, glutamic acid, and aspartic acid can be rapidly administered in large amounts while the administration of casein hydrolysates may be followed by undesirable reactions. Few of these have been serious, but recently an instance of fatal reaction to a casein-pork pancreas hydrolysate has been reported.⁶ Reactions were common with earlier preparations and have been ascribed in part to the manufacturing process.^{5,9,18} Therefore, a reinvestigation

TABLE 1.—"ESSENTIAL AMINO ACIDS" IN CASEIN AND CASEIN DIGESTS (%)

Amino acid	Requirements for rats (Rose)	Casein		"Parenamine"†	"Amigen"‡
		S*	B.B†		
Lysine	1 0	6 3	7 5	8 2	5 8
Leucine . .	0 8	9 7	12 1	10 6	13 5
Phenylalanine	0 7 (dl)	7 9	5 2	3 6	5 6
Valine	0 7	4 0	7 0	7 3	5 0
Threonine	0 5	3 3	3 9	2 9	4 5
Methionine	0 6 (dl)	. .	3 5	3 6	3 0
Isoleucine	0 5	2 5	6 5	6 1	4 8
Histidine	0 4 (dl)	3 8	2 5	1 6	2 0
Arginine .	0 2	1 2	4.1	3 8	5 5
Tryptophane . . .	0 2 (dl)	2 2	1.2	1 0 (dl)	1 0

* Data given by C. L. A. Schmidt.³²

† B.B. data given by Block and Bolling.²

‡ Data supplied by manufacturer.

Amino acids marked (dl) are present in their racemic form.

essential" amino acids. Table 1 lists the contents of essential amino acids of 2 commercial preparations as compared with the minimum requirements given for rats by Rose.²⁹ Minimum requirements for man have not been definitely established. No detailed information is at present available as to the content of the remaining amino acids in these mixtures, which presumably approximate that of casein itself.

of the tolerance of amino acid mixtures prepared with newer methods appeared to be indicated.

The following report discusses the reactions which occurred in the course of intravenous administration of an acid hydrolysate of casein ("parenamine") which when fortified with tryptophane contains all amino acids present in casein. The manufacturing process of parenamine has been changed in recent years³⁰ and

* The study was supported by a grant made to Eloise Hospital by the Frederick Stearns & Co., Detroit, Michigan.

conclusions drawn from observations previously reported are not necessarily applicable to the present mixture. The solution was administered for the express purpose of determining possible hazards inherent in their therapeutic use.

Method. All solutions used were tested before for pyrogenic activity in rabbits, and were found free of such contaminants.* Amino acids from 149 factory lots were tested and care was taken to avoid contamination of the final solutions. The rubber caps of 2 bottles of the 15% mixture supplied by the manufacturer were removed without touching the glass rim of the bottle. In a similar manner a flask containing 500 ml. of 5% glucose in saline, 5% glucose in water or normal saline was opened and 25 or 50 ml. of the amino acid solution were transferred from each of the bottles into the flask. Usually, the total fluid volume was adjusted to 500 ml. by pouring out some of the dilution fluid before the amino acids were added. Occasionally other concentrations were made in a comparable manner. The final concentration of amino acids varied from 1.5% to 7%, 4% being isotonic.³⁰ As only the immediate reaction to amino acids was of interest the type of dilution fluid was of no importance. The usual practice of giving large amounts of glucose simultaneously to avoid excessive neoglycogenesis³ was therefore not always followed. The final mixture contained amino acids from 2 different bottles of the same lot, occasionally from 2 bottles of 2 different lots.

Patients were chosen at random from various services of the hospital, the only prerequisite being the absence of any fever for at least 5 days prior to the testing. Some exceptions are noted below. The infusions were started after a base line for temperature, pulse and respiration had been established. The patients were checked at $\frac{1}{2}$ -hourly and hourly intervals for 4 or 5 hours after the end of the infusion or until any reaction had completely subsided. With few exceptions all patients received 2 infusions, 1 containing 50 ml. (7.5 gm.) and 1 100 ml. (15 gm.) of the original mixture. The same lot at the same concentration was always given to at least 2 individuals simul-

taneously. Therefore, reactions occurring in only 1 of the simultaneously tested patients could reasonably not be ascribed to the amino acid mixture itself. However, if such a reaction did occur, series of similar concentrations from the same lot were further tested in other individuals. One lot was, therefore, tested on from 2 to 6 individuals before judgment as to its purity was passed.

The speed of the infusion was adjusted so that 500 ml. of fluid were delivered in from 45 to 90 minutes (average, 60 minutes). This proved difficult at times and occasionally an infusion would be completed in less than 30 minutes or in more than 2 hours.

In a limited number of cases blood amino acid levels were determined before, during and after the infusion. A micro-method for determination of capillary amino acid nitrogen was used, based on the color reaction of amino nitrogen with naphtho-quinone sulfonate as described by Folin and modified by Sayhun and by Krauel.²⁰ Values taken on normal subjects 2 hours after a light breakfast ranged from 7.1 to 8.7 mg. per 100 ml. (mean 7.7). These figures are higher than those obtained by others using the ninhydrin method.^{35,38} The difference is explained by the fact that whole blood rather than plasma was used. In addition the naphtho-quinone method does not measure amino acids alone. In fact, compounds other than amino acids may react with quinone to a certain extent.¹⁴ Such considerations, however, were thought to be immaterial as the object of the investigation was merely to measure rough changes over a relatively short period of time in fasting patients after a known amount of amino acids had been added to their blood.

Results. Five hundred and fifty infusions were given to 303 patients. As seen from Table 2, 22 reactions were observed in 21 patients, that is, 7% of all subjects experienced a reaction of some kind (4% of all infusions given). In 4 patients (1.3%) the infusion had to be discontinued because of the severity of the reaction. No reaction, however, was alarming or was followed by long-standing or permanent changes.

Table 2 shows clearly that the first

* The data on pyrogen tests were kindly supplied by Dr. C. W. Geiter, Frederick Stearns & Co., Detroit, Michigan.

2 types of reactions which occurred in 8 cases, namely, fever with or without chills, may be grouped together. There is no striking difference in the final amino acid concentration given or in the average infusion time. Such febrile reactions occurred in 1.5% of all infusions and constituted 36% of all reactions encountered. In a number of instances, such reactions could be traced directly to accidental contamination of the infusion set.

In the second patient tested simultaneously and with the same mixture, a temperature rise to 99.6° F. was noted 4 hours after the end of the infusion. No chill occurred. When the final mixture for these 2 patients had been prepared it was left standing in a warm and well-lighted room for over 2 hours before the infusion was started. The same lot was retested in 1 of the 2 patients and in 2 other individuals and no reactions were encountered.

TABLE 2.—22 REACTIONS ENCOUNTERED DURING 550 INFUSIONS OF AMINO ACIDS (PARENAMINE)

Symptoms	No. cases	Average infusion time (min.)	Average amino acid concentration (%)	% of all reactions encountered	% of all infusions
Chills and fever	4	53	3.6	18	0.7
Fever without chills	4	55	2.4	18	0.7
Nausea with or without vomiting and hot flashes	8	36*	3.0	36	1.5
Severe headache	2	..	3.0	9	0.4
Precordial pressure	1	50	3.0	5	0.2
Lumbar pain	1	40	1.5	5	0.2
Dyspnea	2	67	4.0	9	0.4
Total	22	100	4.1

* One patient suffered from nausea without vomiting the 1st day (infusion time 130 minutes; not calculated in this table) and severe headache the 2nd day. (See text.)

A patient with bronchogenic carcinoma and extensive atelectasis showed a rise in temperature from 99° to 104° after an injection of 500 ml. of 1.5% solution in 20 minutes. Four other patients tested with the same lot had no reaction. It was noted in this case that due to some irregularities in setting up the solution the needle adapter was handled manually in a manner which made secondary contamination possible. Considerable handling of the adapter of the infusion set due to a plugged needle was necessary in another patient who experienced a sudden chill with a rise of temperature to 102° F. after injection of 200 ml. of a 3% amino acid mixture in glucose. Six of these febrile reactions occurred singly and the patients tested simultaneously experienced no abnormal reactions. Once a simultaneous reaction occurred in 2 individuals. The first experienced chills and fever up to 102° F. with extensive diaphoresis maximal 2 hours after the end of the infusion.

At other times no obvious reason for such febrile reactions could be found. In 2 patients suffering from tuberculosis a definite rise in temperature apparently directly related to the infusion was noted. A patient with pulmonary tuberculosis received a 3% solution in 5% glucose and saline over 45 minutes. He experienced a rise in temperature without chills reaching a peak of 102° F. within 2 hours after the end of the infusion. In 3 other cases of tuberculosis simultaneously tested with the same solution no reaction was observed. Another patient with tuberculosis of the spine and transverse myelitis experienced a temperature rise to 102° F. after an infusion of a 1.5% solution in 5% glucose. Five other patients experienced no symptoms with the same batch of amino acids. In this instance the febrile response could be repeated several days later when a 3% solution made from a different batch was offered within 30 minutes.

Another distinct type of reaction is characterized by flushing of the face, nausea and dizziness often associated with an explosive type of vomiting. In all but 1 of the 8 cases of this group the infusion was offered rapidly with an average infusion time of only 36 minutes as compared to 60 minutes infusion time seen with most of the other reactions (Table 2).

into a definite pattern. In 4 cases severe headaches, precordial constriction and vague body pains, particularly noticeable in the lumbar region, were observed. In 1 of the patients, a sharp occipital headache occurred after the infusion bottle was accidentally shaken violently before the infusion was started. Two patients with severe anemia and atherosclerotic

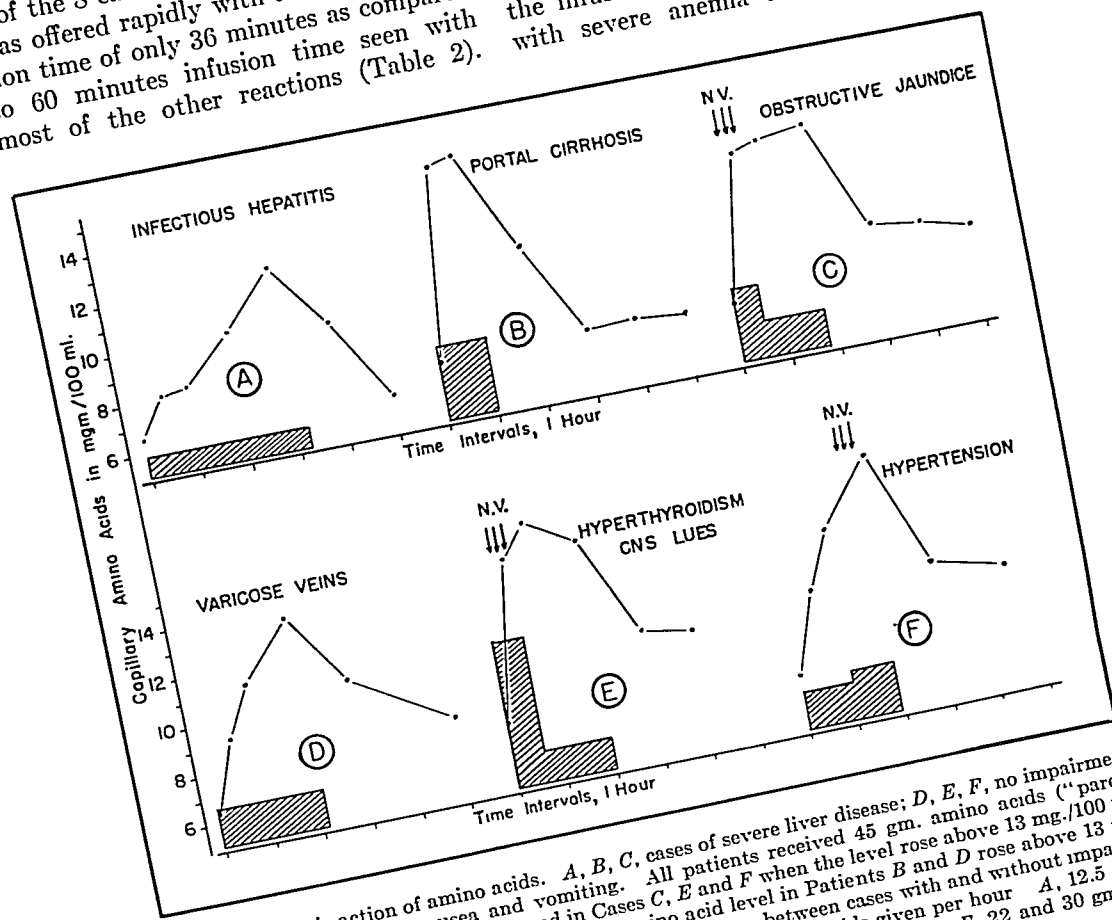


FIG. 1.—Emetic action of amino acids. A, B, C, cases of severe liver disease; D, E, F, no impairment of liver functions; N, V, nausea and vomiting. All patients received 45 gm. amino acids ("parentamine") intravenously. Nausea occurred in Cases C, E and F when the level rose above 13 mg./100 ml. It was observed at lower levels in others. The amino acid level in Patients B and D rose above 13 mg. without reaction. Note that there is no appreciable difference between cases with and without impaired liver function. The hatched squares indicate the amount of amino acids given per hour: A, 12.5 gm./hr.; B, 45 gm./hr.; C, 45 and 22 gm./hr.; D, 22 gm./hr.; E, 90 and 22 gm./hr.; F, 22 and 30 gm./hr.

The administration of amino acids proceeded almost twice as fast in this group as compared to others while the average concentration of the final solution did not differ from the rest. In some instances the symptoms disappeared when the speed of the infusion was reduced or a new infusion started at a slower rate (Fig. 1, C, E). The other reactions noted do not fall

heart disease experienced dyspnea at the end of the infusion, in 1 associated with an excessive sinus tachycardia.

Discussion and Further Observations. Of the amino acid infusions of this series, 1.5% were followed by a febrile response with or without chills. None was serious and all subsided within a few hours. Febrile reactions are relatively common in

occurrence whenever parenteral fluids are being administered. Recent reports on plasma, whole blood, or red cell transfusions show an incidence of approximately 2%.^{7,11,35} A recent report on the reaction incidence of a red cell-glucose-amino acid infusion mixture lists 3.8% febrile reaction.³² In some series, such reactions were encountered in as high as 10%¹ and 14%⁸ of all transfusions given. Pyrexial reactions of this type are usually attributed to foreign protein and bacterial disintegration products which contaminate the fluid administered, tubing, glassware or needles used. In a series of 889 blood transfusions observed during 1930 and 1931 Lewisohn and Rosenthal reported a decline of febrile reactions when scrupulous technique in cleaning of instruments and tubing was instituted after the first 412 cases.²² In the present series some of the reactions obviously could have been avoided but even so the incidence of febrile reactions (1.5%) does not appear higher than in comparable series of plasma or blood transfusions.

Febrile reactions are more likely to occur in severely anemic patients or in those suffering from infections of various kinds.^{11,27} Some of the cases cited above seem to bear this out and a specific action of amino acids on the thermoregulatory centers cannot be excluded particularly when reactions of this type occur regularly and repeatedly in 1 case and not in others. Injections of crystallized amino acids in large amounts have resulted in febrile reactions in one series of cases.³³ That standing of a ready made-up infusion may result in febrile reactions has been reported³⁵ and was found once in the present series. A small number of bacteria in the infusion set-up may not interfere with the infusion, but further growth upon standing may be sufficient to elicit a febrile response. One should remember that an isotonic amino acid glucose mixture has been shown to be a perfect culture medium for bacterial growth.^{4,21}

Overloading of the circulation by sud-

denly increasing the circulating fluid volume in the face of anemia and heart disease or both, is likewise known as an avoidable complication of transfusions in general.^{37,39} A similar reaction was observed twice in the present study and could have been prevented by slowing of the infusion or by constant drip administration.

The importance of so-called nitritoid reactions following parenteral fluid administrations has recently been stressed by Strumia, McGraw and Blake.³⁵ Such reactions consisted in a sense of constriction of the chest, pain in the lumbar region, headaches and occasional nausea and vomiting. Four instances somewhat reminiscent of the syndrome were observed in the present series. In 1 instance nausea without vomiting occurred during the latter part and shortly after an infusion of 3% amino acids had been offered over a period of 130 minutes. The next day this patient reacted with a very severe headache when a 6% solution was offered within 125 minutes. No reactions were noted in a second patient who received the same solution simultaneously. It is felt that this patient might have undergone a nitritoid reaction. The nausea observed on the first trial was obviously not caused by rapid infusion responsible for a similar symptom in 7 other cases.

All these reactions are familiar wherever infusions of any type are given. Amino acid mixtures are not specifically responsible for them and they do not appear with greater frequency when amino acids instead of crystalloids or blood or plasma are being given by parenteral route.

On the other hand, hot flashes, nausea and vomiting which follow rapid administration of casein hydrolysates have been noted by several observers and they appear to be characteristic of reactions to such mixtures of amino acids. From Table 2 and from statements made by others, it appears obvious that these reactions have some relation to the speed with which the solution is being offered. This

is further suggested by the fact that these symptoms disappear soon after the end of the infusion or when the speed of inflow is reduced. A simple explanation suggests itself immediately: amino acids are re-synthesized primarily in the liver and a

of inflow to the rate of removal. If this assumption is correct, hyperamino-acidemia should be present in cases showing nausea but not in others or at least blood amino acid levels in these reactors should be considerable in excess of those found

TABLE 3.—AMINO ACID LEVELS OF CAPILLARY BLOOD AT INTERVALS FOLLOWING INFUSION OF 45 GM. AMINO ACIDS (PARENAMINE) (MG./100 ML)

No. cases	Reactions	Fasting	30 min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	7 hrs.
7	None	7 3	11 0	11 6	12 7	10 4	9 2	8 5
5	Nausea, vomiting, hot flashes	7 3	10 5	12 6	13 1	11 2	9 4	8 7

TABLE 4.—AMINO ACID LEVELS OF CAPILLARY BLOOD FOLLOWING INFUSIONS OF 45 GM. AMINO ACIDS (PARENAMINE): CASES OF NORMAL AND ABNORMAL LIVER FUNCTION COMPARED (MG./100 ML.)

No. cases	Type and reaction	Fasting	30 min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	7 hrs.
3	Normal,* no reaction	8 0	10 9	11 6	13 4	11 2	10 1	9.0
4	Normal, nausea, etc.	7 2	10 1	12 3	12 9	11 8	9 5	8.8
4	Abnormal,† no reaction	6 8	11 4	11 6	12 4	9 8	9 0	8.0
3	Abnormal, nausea, etc.	7 4	13 2	13 5	13 8	9 4	9 1	8 4
		6 9‡	10 3	10.3	10 3	8 1	6 5	6 5

* Normal: Dermatitis, varicose veins, paresis, hypertens on, bronchitis, bronchogenic carcinoma, mild toxic goiter.

† Abnormal: Portal cirrhosis of liver (5 cases), silent stone with obstructive jaundice, catarrhal jaundice.

‡ One patient with cirrhosis who received only 30 gm. amino acids (i.v.). Nausea and vomiting occurred during the 2nd hour of administration.

TABLE 5.—AMINO ACID LEVELS OF CAPILLARY BLOOD FOLLOWING RAPID INJECTION OF 7.5 GM. AMINO ACIDS (PARENAMINE)

No. cases	Type	Injection time, min.	Fasting	10 min.	30 min.	40 min.	50 min.	60 min.
1	Normal	10 0	8 8	..	7 8	7 5	7.5	7.9
2	Cirrhosis	10 0	8 2	..	8.7	9 5	9 4	8 4
3	Normal	5 0	7.8	12 4	10.7	9 3	9 1	7 9
5	Cirrhosis	5 0	7 5	12 3	10 2	8 6	9 1	9 1
1	Inf. hepatitis	5 0	8 1	12 5	10 4	8 5	9 5	7 4
1	Normal	2 5	7 8	14 8	11 3	9 6	8 1	8 4
2	Cirrhosis	2 5	8 5	12 8	11 3	9 9	9 0	8 9
1	Inf. hepatitis	2 5	7 9	10 9	10 0	7 8	..	7 9

No reactions occurred in any of the cases.

No rise of amino acid levels was observed when 7.5 gm. amino acids were offered within 10 minutes, apparently indicating that removal of amino acids from the blood stream occurred at a rate exceeding 0.75 per minute in the normal individual as well as in 2 cases of advanced portal cirrhosis (biopsy).

discrepancy between the speed which such products are offered and the capacity of the liver to remove them from the blood stream might conceivably be a factor causing nausea and vomiting. This is quickly relieved by adjusting the speed in persons not suffering from nausea and vomiting. Secondly, patients suffering from diseases of the liver with marked impairment of function would be expected to tolerate amino acids much less readily than normal individuals, would show a

higher degree of amino-acidemia and might present a higher incidence of nausea and vomiting. Some evidence can be cited in support of this thesis. It has been stated that amino acids are not tolerated when icterus is present.^{3,28} Reactions are said to be common in infectious hepatitis.¹⁹ On the basis of the assumption mentioned above, cirrhosis of the liver has once been mentioned as a contraindication to parenteral amino acid administration.³ Liver damage was said to be present in the fatal case reported by Curreri, Hibma and Cohen,⁶ and was stressed as a complication by Hopps.¹⁶ A decrease in plasma clearance of amino acids in hypoproteinemic dogs and in patients with diseases of the liver has been reported.^{15,23} None of these statements, however, have gone unchallenged and in fact amino acid solutions have been used extensively in the treatment of cirrhosis of the liver^{12,13,19} and in infectious hepatitis.³⁴

In an attempt to obtain some information relative to the cause of this reaction amino acid levels were determined by measurements of capillary blood in a number of cases before, during and after parenteral infusion of amino acids. Of the 14 cases thus tested, 7 were patients suffering from a variety of diseases not severe enough to affect liver function appreciably, 5 had advanced portal cirrhosis confirmed by biopsy, 1 had severe obstructive jaundice of long standing (silent stone) and 1 had infectious hepatitis. All but 2 patients thus tested received 45 gm. of amino acids diluted to 1000 ml. of fluid with 10% glucose (4.5% solution). They were fasting and received no food for 7 hours after the beginning of the infusion. Table 3 reveals the absence of any significant differences in the amino acid levels in cases reacting with nausea and vomiting and in those tolerating the infusion. Furthermore, there appears to be no striking difference between cases with and without diseases of the liver, as indicated in Table 4. Although only a few cases were tested in this manner the results

were consistent and the ranges of the amino acid concentration in the individual cases were not striking. However, from the inspection of some of the curves it appeared that the speed with which a higher amino acid level was obtained rather than the level itself may have been one factor responsible for the reactions (Fig. 1, *C*, *E*). A rapid rise was observed in other cases who did not suffer emesis (Fig. 1, *B*). It was found, for instance, that nausea and vomiting would occur when the level rose from 7.4 to 13.2 mg. % It subsided promptly when the speed of the infusion was reduced, although during the next 90 minutes the level rose from 13.2 to 13.5 and from 13.5 to 13.8 mg. % (Fig. 1, *C*). (The differences may lie within the error of measurement.) In another case nausea and explosive vomiting was present when the amino acid level ascended from 7.7 to 14.2 mg. within 30 minutes. When the inflow was adjusted the level rose still further to 15.5 mg. during the next hour but nausea subsided (Fig. 1, *E*). It was, therefore, surprising to observe that when amino acids were offered very rapidly with a syringe in 15 cases, not a single instance of nausea and vomiting occurred although steep rises in amino acid levels were present (Table 5). Again, in the small number of cases no significant differences were noted between cases with normal and abnormal liver function. Obviously, the speed of administration must be of some importance as repeated observations have shown that slowing of the infusion will prevent such reactions. Rapid administration of amino acids by syringe is necessarily limited as to the total amount offered and not more than 7.5 gm. parentamine was given in each case. The blood levels obtained, however, were comparable to those observed in patients receiving larger amounts over longer periods. In addition to the speed of administration one might assume that saturation of the liver with amino acids might be another factor concerned in the development of

nausea and vomiting. It appears, then, that the rapidity of the rise of amino acid concentration in the blood plus the total amount offered are the two important factors responsible for the reactions encountered.

It has recently been reported that certain amino acids, particularly glycine, glutamic acid and aspartic acid, may be responsible for intolerance.^{24,25,26} In hypoproteinemic dogs no reactions occurred, in spite of extremely rapid administration of fairly large amounts when these substances were omitted from crystalline amino acid mixtures. In these series of experiments mixtures of crystallized amino acids were uniformly better tolerated than mixtures prepared from casein hydrolysates. This might be explained by the fact that in casein glutamic acid is present in amounts over twice as high as any other individual amino acid.³¹ Our observations on total amino acid levels during and after infusions neither confirm nor deny these findings, but the fact that no correlation of the total amino acid level to intolerance was found would favor the concept that individual components of the mixture might be responsible for such reactions as hot flashes, nausea and vomiting. The amount of glutamic acid offered on rapid injection in the examples presented might not have been sufficient to reach a reaction threshold.

Another type of reaction which is regularly mentioned as typically associated with amino acid administration is the tendency of such mixtures to cause phlebotrombosis. This has been encountered frequently by us during the routine use of amino acid hydrolysates, although in the present study thrombosis was not encountered, because the time of infusion was short and the administration not repetitive. Both commercial preparations have a pH of about 4. When diluted to a 4% solution the mixture is equal in isotonicity to normal saline or 5% glucose. It has recently been stated that phlebitis is caused by the hypertonicity of the solu-

tion rather than by the amino acids themselves.¹⁷ No more than 45 gm. of amino acids is usually given in 1000 ml. of a dilution fluid so that the assumption that phlebotrombosis is caused by the hypertonicity of the solution is rarely met under ordinary circumstances. It might again be possible that individual amino acids by themselves or by locally combining with other substances may be concerned in endothelial damage and intravascular clotting. Acidity of the final solution would hardly be a factor in the ordinary dilutions used.

Conclusions. The results of this study in general are in accord with the experiences of those who have used amino acids before. There is a definite but rather low incidence of reactions. Some are seen with any type of parenteral fluid administration, others are characteristic for solutions of amino acids. It appears justified to follow certain precautions when mixtures of amino acids of the types at present available are to be given parenterally:

1. In preparing the final solution utmost care should be taken to avoid accidental contamination in mixing or in starting the infusion. Glucose protein mixtures of the type used are perfect media for bacterial growth. The mixture should, therefore, be prepared by the physician who plans to use it and the procedure of dilution should not be left to the nursing staff. The recent introduction of a ready made solution of amino acids in glucose should go far to prevent febrile reaction caused by improper handling.

2. Once set up in the final dilution, the mixture should be given without delay.

3. The speed of flow should be adjusted so that not more than 15 gm. hydrolysate (100 ml. parenamine) are offered per hour (0.25 gm. per minute).

4. As endothelial damage and phlebotrombosis cannot be avoided when amino acids are to be given over a long period of time, a vein on the dorsum of the hand should be used and subsequent injections

should be given into the same vein, gradually moving the point of insertion proximally. Thus the vein can be used even if its distal end has become sclerosed and multiple thromboses of superficial veins can be kept to a minimum.

If these precautions would have been followed at all times during the present series, the incidence of reactions could have been lowered to at least 10 instead of 22 cases and uneventful infusion would have been observed in 98% instead of 93% of the cases. The incidence of pyrogenic reaction in this series was not greater than in any of the reported series of blood and plasma transfusions.

Summary. 1. Twenty-two reactions were observed in a series of 550 infusions of an amino acid solution (casein hydrolysate) given for the sole purpose of determining the incidence and character of reactions inherent to parenteral protein alimentation. The reaction incidence was 4%.

2. Eight of these reactions consisted of sudden onset of fever with and without chills (1.5% of all infusions). It does not seem justified to attribute the pyrogenic reactions to the acid hydrolysate admin-

istered. In a number of cases accidental contamination appeared likely, in others tubing reactions might have been responsible. In still others, a rise in temperature was noted without an apparent exciting cause.

3. Nausea with or without vomiting may occur when the speed of administration exceeds 15 gm. per hour. Blood amino acid levels taken during this period showed little difference when those reacting with nausea and those tolerating the infusion were compared. It is likely that the steepness of ascent of amino acid levels plus the total amount of amino acids offered were responsible for the reactions of this type. These findings favor the view that some individual components of the mixture may be responsible for nausea and vomiting produced by solutions of this type.

4. Under conditions of these experiments, cases with diseases of the liver did not appear to react differently as compared with others.

5. Certain recommendations are made which, if followed, should reduce reactions to parenteral amino acid administration to the unavoidable minimum.

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MALARIAL JAUNDICE

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JAUNDICE is known to occur in malaria,⁸ as indeed is hardly surprising in a hemolytic disease, but it is uncommon. The clinical records of the 20th General Hospital, a United States Army installation located in Assam Province in India, make available for study 8837 cases of malaria occurring between May 1, 1943, and Sept. 1, 1945; and it has been thought worth while to review this material to the end of elucidating the clinical syndrome of malarial jaundice.

Method of Study. The records of the clinical laboratory were scrutinized and all reports (321) submitted by the laboratory of a quantitative van den Bergh exceeding 0.6 mg. % or an icterus index exceeding 10 were collected.* The clinical records of all these patients were then inspected and all those which showed the occurrence of malaria at or near the time the patients were jaundiced were put aside for study. These charts numbered 30 and comprised the available case records of all our patients who had jaundice in close association with malaria during the period covered by this study.

It soon became apparent that 24 of the 30 patients exhibited a unified clinical syndrome which unquestionably represented malarial jaundice. Briefly this was characterized by the sudden onset of painless hemolytic jaundice of varying degree during an attack of malaria, in all instances while the patient was still febrile, which subsided rapidly and completely, and which was perfectly benign provided it was uncomplicated.

Six of the 30 records were excluded from the study. Two were those of patients treated elsewhere for malaria and transferred here afebrile and asymptomatic, each of whom had a slight elevation of serum bilirubin on admission (van den

Bergh 0.9 mg. and 0.8 mg. per 100 cc., respectively). These patients may have had malarial jaundice, but we have insufficient data to draw any conclusions. Another patient developed falciparum malaria on the 11th day of a perfectly typical catarrhal jaundice. The other 3 patients became jaundiced either during a malarial attack or (2 cases) 1 to 3 days thereafter; but the long duration of the jaundice, and the onset of the disease, with constipation and dull abdominal distress, clearly demarcated it from the syndrome of malarial jaundice; and in addition their jaundice was not of the hemolytic type, as none was anemic and the 2 who had more than one hemoglobin estimation showed no fall during their disease.

Analysis of Cases. Table 1 records the clinical and laboratory data in the 24 cases of malarial jaundice studied. The admission histories (with the exception of Case 8, in whom no adequate history was obtainable) recounted in general the banal symptoms of an acute attack of malaria. Unusual features in any patient's record are noted in Table 1 and in the appended comment.

Comment. 1. *Age, Race and Incidence.* Malarial jaundice appeared in 0.27% of our patients with malaria (24 of 8837). All were soldiers of the Chinese or American Army, and only 7 were older than the 3rd decade. Table 2 shows percentage calculations based upon nationality and parasite species. Malarial jaundice was about 4 times more common in falciparum than in vivax malaria; but nationality did not appear to influence its incidence. Jaundice was not seen in 100 cases of quartan malaria.

* Acknowledgments are due Captains H. H. Hodges and William Fitts, A.U.S., who did this portion of the work.

TABLE I.—ANALYSES OF DATA OF 24 CASES OF MALARIAL JAUNDICE

Case No.	Age, Race	Prior med.	Abdominal	M.P. smear	van den Bergh	Icterus index	Jaundice		Hemoglobin	Urine	Liver	Spleen	Com- plica- tion	Treat- ment	Comment
							Duration								
1	22 Ch	?	0	F +++	26 (8th) 6 (12th)	+	5th) 0 (11th)	12.5 (5th) 10.5 (8th)	B: 0 (8th)	+	+	?	Q A	
2	25 Ch	?	0	F +++	43 (5th) 7 (11th)	+	4th) 6th) 8th) 0 (11th)	14.0 (4th) 13.2 (5th)	Dark (4th) B: 0 (5th)	0	0	0	Q A	
3	19 Ch	Q A	0	F ++	...	28 (5th) 7 (9th)	14.2 (4th) 11.2 (9th)	0	0	0	Q A	
4	21 Ch	0	0	F +++	14 (8th)	+	5th) 8th) 0 (11th)	10.2 (5th) 9.2 (7th) 7.5 (10th) 9.2 (24th)	0	+	?	A C	Stool + B (8th)
5	29 Ch	0	0	V +++	17 (9th)	0	8th) 9th) 0 (17th)	15.0 (5th) 11.5 (9th) 11.0 (14th)	+	+	0	Q A	Stool + B and (8th and 12th)
6	47 W	Q A	0	F	1.6 (9th)	18 (9th)	+	(7th)	12.3 (8th) 10.8 (12th)	+	0	0	A	
7	30 Ch	?	?	F +++	35 (7th)	+	(6th)	8.4 (7th)	B: + (6th) B: + (7th) Ur: + (7th)	0	+	C	Q A	Died
8	27 W	?	0	F +++	5.0 2nd Hosp. Day	+	1st HD 2nd HD	Orange: (1st) B: + (2nd) Hb: 0 (3rd)	+	0	C	Q A	Died
9	20 Ch	Q A	0	F +++	21 (7th) 20 (8th)	+	(7th) 10th) 0 (12th)	17.0 (3rd)	Dark (8th) B: 0 (9th) Ur: (9th)	+	+	0	Q A	
10	28 Ch	Q A	0	F +++	1.8 (6th)	22 (6th)	+	(5th) 0 (by 17th)	7.1 (5th) 7.2 (6th) 0.6 (17th)	0	+	?	Q A	

2. *Abdominal Symptoms.* Though nausea and vomiting were not uncommon, abdominal distress of any type occurred in only 3 patients, all of whom had falciparum malaria. Two of them (Cases 22 and 23) complained of cramping pains, quite unlike the dull distress of catarrhal jaundice; and both these soldiers were passing, at the time, frequent watery scanty stools which contained no blood or pus. This syndrome was not rare in our malarial patients and was thought to be a mild malarial dysentery. The third patient (Case 24) had fairly severe steady right upper quadrant pain unassociated with diarrhea.

quently was fairly accurately determined. In the 13 patients in whom jaundice was present on admission, the day of onset was taken to be the day of admission, thereby probably introducing a slight error. On the other hand, the date of the disappearance of clinical jaundice was not always evident from the record. A normal or practically normal laboratory determination of serum bilirubin was utilized in fixing the limit of jaundice where one could be sure that the jaundice had not disappeared more than a day or 2 prior to the normal reading. In 8 patients, information on this point was insufficiently accurate, and no calculation has been

TABLE 2.—RATIO OF MALARIAL JAUNDICE TO TOTAL MALARIA BY GROUPS

	Chinese	American	Total
Vivax	$\frac{2}{1361} = 0.14\%$	$\frac{3}{2605} = 0.11\%$	$\frac{5}{3966} = 0.12\%$
Falciparum	$\frac{13}{3300} = 0.4\%$	$\frac{6}{1471} = 0.4\%$	$\frac{19}{4771} = 0.4\%$

3. *Smear for Malaria Parasites.* Five patients had trophozoites of *P. vivax* in the blood smear; 18 showed trophozoites of *P. falciparum*. No mixed infections occurred. One patient (Case 17) showed a plasmodium the species of which was undetermined; his disease has been considered to be falciparum malaria because of the clinical phenomena of severe dizziness, so common in falciparum malaria, and sustained high fever. Two seriously ill patients (Cases 8 and 24) showed also quantities of segmenting forms of *P. falciparum* in the peripheral blood. One patient (Case 19) showed numerous merozoites of *P. vivax*.

As was to be expected, *P. falciparum* gave rise in general to a more severe illness than did *P. vivax*. All 3 fatalities were due to *P. falciparum* infections, as were the more severe grades of jaundice. *P. malariae* was demonstrated in none of these patients.

4. *Jaundice.* The calculation of the mean duration of jaundice is subject to several inaccuracies. The day of appearance of icterus in 11 instances was subsequent to the day of admission, and conse-

made of the duration of their jaundice. The 3 patients who died while still jaundiced also did not figure in the estimations of the duration of icterus.

The average duration of jaundice as calculated in the remaining patients was 7 days, with a maximum of 15 (Case 17) and a minimum of 2 (Case 14). The average day of onset was the 4th day of fever, and varied between the 1st and the 7th.

The fact that our patients had serum bilirubin determinations by 2 different techniques introduced a problem in the evaluation of the average maximal bilirubinemia, since it is not possible to assume an accurate equivalent icterus index for a given van den Bergh reading, or *vice versa*. However, by counting only one of the laboratory methods in each of the 5 patients who had serum bilirubin estimations by both techniques—the icterus index in Cases 17 and 21 and the van den Bergh in Cases 6, 10 and 16—an average maximal van den Bergh of 2.4 mg. per 100 cc. was obtained in 10 patients, and an average maximal icteric index of 27 in the other 14 pa-

tients. Since these figures are roughly equivalent, it is felt that the calculation is substantially as accurate as it would have been had only one method been used. There remains, of course, the error introduced by the unlikelihood that blood samples were taken at the height of the jaundice in all cases.

The qualitative van den Bergh reactions have not been tabulated. All the sera which gave a very high quantitative titer showed a strong direct reaction. No immediate reaction was obtained when there was only slight quantitative elevation. The intermediate quantitative readings were accompanied by biphasic qualitative reactions.

5. *Hemoglobin*. In only 6 of our patients were hemoglobin determinations made which spanned the period during which the icterus was waxing (Cases 1 to 6). In all 6 there was a fall in hemoglobin compatible with rapid destruction of red blood cells. The mean fall measured 2.25 gm. % measured over a mean time interval of 3.7 days. The determinations were not systematic enough to yield information concerning the relative quantity of erythrocyte lysis in any given patient; what they do show is that in all 6 cases definite blood destruction accompanied the rising serum bilirubin; and one may safely conclude that their jaundice was of the hemolytic variety.

There is every reason to suppose that the other patients' jaundice was on the same basis. Many of them were admitted already jaundiced and, as would be expected, their admission blood counts almost always showed a rather marked anemia. An occasional patient (Cases 9 and 23) had a single reading 3 to 4 days before jaundice appeared, and they showed no anemia. Only 1 patient (Case 17) had a single normal reading at a time when he was becoming jaundiced.

It is concluded that malarial jaundice is of the hemolytic variety.

6. *Urine*. (a) *Bile pigments*: The urine of 11 of these patients was not tested for the presence of bile pigments.

Of the remaining 13 patients, 9 passed urine containing bile by chemical test. The laboratory returned a negative report on the urines of the 4 remaining patients. However, 2 of these men had passed a dark colored urine the day before the specimen examined by the laboratory was collected. Apparently bilirubinuria is the rule in this syndrome.

(b) *Hemoglobinuria*: Three patients passed free hemoglobin in their urine. In 2 of them (Cases 20 and 24) the characteristic reddish color was present and the benzidine test was positive; in the 3rd (Case 17) no mention is made of the color, but the benzidine test was positive on a urine which contained no red blood cells. A 4th patient (Case 8) probably had hemoglobinuria; his urine was described as "brownish orange," but no benzidine test was done until the 2nd day, at which time the urine was no longer reddish but dark brown, and was benzidine-negative, though positive for bile. This patient is the only one of whom it is specifically stated that no antimalarial drug had been taken prior to the appearance of reddish urine.

The prime importance of the fact that *these 4 patients had the highest elevations of serum bilirubin* in our whole series will be further discussed.

7. *Stools*. Stool pigment has not been tabulated because it is mentioned specifically in only 2 patients, both of whose feces contained bile. It is quite unlikely that acholic stools passed by febrile jaundiced bed patients would escape the attention of the ward officer. Presumably most of the stools contained excess bile.

8. *Liver and Spleen*. The liver was enlarged in 10 of our cases, in 3 of which it was tender. The spleen was palpable in 13.

9. *Complications*. (a) *Cerebral malaria*: A number of patients, without exhibiting full-blown cerebral malaria, gave evidence of mild central nervous system irritation. Those who, in addition to severe headache, had any of the phenomena of severe dizziness, stiff neck, mental dullness, or reflex

changes are tabulated as questionable cerebral malaria.

Three patients (Cases 7, 8 and 24) had undoubted cerebral malaria; and in all the disease ended fatally. All were stuporous to unconscious throughout their hospital course (3 to 4 days); and all had reflex abnormalities and positive Babinski signs. Two (Cases 8 and 24) expired in deep coma, the other after 2 severe generalized convulsions.

Cerebral involvement appears to bear no relation to icterus in malaria. The 3 cases of cerebral malaria represent a small fraction of the total number of patients with that disease seen here during the period covered by this study, and of course none of the others was jaundiced.

(b) Azotemia: Two patients developed a severe acute azotemia as manifested by B.U.N. determinations of 105 and 113, respectively. One of them (Case 20) entered the hospital deeply jaundiced and in stupor, but he was vigorously treated with quinine and atabrine and his fever subsided. He had transient hypertension. Although his icteric index and B.U.N. were normal after 3 weeks, he was still unable to concentrate his urine above 1.012.

The other patient (Case 24) was one of the 3 cases of cerebral malaria. He was admitted in stupor, sank slowly into deeper coma, and expired quietly on the 3rd hospital day. It seems probable that the cause of death was cerebral, although nitrogen retention, measured on the 2nd day, was marked.

It is of interest that these 2 azotemic patients constitute 2 of the 3 patients who had definite hemoglobinuria. That the association is more than coincidental is further suggested by the fact that the patient (Case 8) who passed brownish orange urine which was not submitted to chemical examination had a B.U.N. of 34; and that no other patient in our series was reported to have a B.U.N. above 22. The subject will be further discussed in a later section of this paper.

10. *Medication.* Because of the possible relationship of malarial jaundice to black-water fever, in which condition quinine has been thought by some to play a pathogenic rôle, and because it is known that atabrine may under some circumstances be hepatotoxic,⁴ both the ingestion of these drugs shortly prior to the appearance of jaundice and their therapeutic exhibition in this hospital have been tabulated. Ten patients had taken neither drug therapeutically prior to the onset of icterus, and only 1 of this group (Case 24) had been receiving suppressive therapy with atabrine. Nine had been treated with atabrine and 7 with quinine. There is therefore no indication that either drug played any part in the production of jaundice.

No patient was treated here with quinine alone. Seven received atabrine alone, and the average duration of their jaundice was 7 days—precisely the same as in those patients who also received quinine. Evidence is lacking to convict either drug of prolonging the jaundice.

It is noteworthy, but probably of no significance, that the shortest-lived jaundice occurred in the 1 patient who was treated with a new antimalarial chemical, SN-6911 (Case 14).

11. *Autopsy Material.* Three patients died (Cases 7, 8 and 24) and postmortem examinations were performed on all. All showed congestion and edema of the brain, liver, kidneys and pancreas. Pigment macrophages crowded the capillaries of all the organs. The livers were grossly firm and congested; the sinusoids were wide; the cords pale, granular and swollen, and contained large amounts of pigment granules (hemosiderin). The microscopic appearance of liver tissue was thought to indicate the presence of hepatic damage.

Discussion. 1. *Hemolysis and Liver Damage.* It is a familiar fact that in hemolytic icterus of any type some impairment of liver function occurs which acts as an accessory in the production of jaundice. Rich⁶ has pointed out that 95% of a dog's liver can be excised with-

out producing jaundice, and has suggested that anoxemia of the hepatic cells resulting from anemia may account for failure of liver function in hemolytic diseases. The microscopic sections of liver tissue in these cases make one speculate whether the flooding of the organ with pigment, not only in the endothelial and Kupffer cells, but in the parenchyma as well, may not have some toxic effect. Using the bromsulfalein excretion test, Machella, Burgoon and Fine,³ working at this hospital, have presented evidence that fever itself may depress hepatic function. They found that in 36 attacks of *P. falciparum* malaria in Chinese soldiers there was abnormal dye retention during the febrile period; and they were able to demonstrate retention of the same order of magnitude in 3 febrile subjects in whom pyrexia was induced artificially by the injection of typhoid vaccine.

Whatever the cause, it is clear that the liver is damaged in malarial jaundice. The sections afford evidence of degenerative changes in the parenchymal cells, and the presence of a direct van den Bergh reaction and of bile in the urine in many instances is indicative of intrahepatic microscopic regurgitation into the blood stream of cholebilirubin.

Serial dye excretion studies in 3 of our mildly jaundiced patients³ show that the hepatic dysfunction is transient, at least in the milder grades of jaundice. Abnormal dye retention 1 week after the temperature returned to normal was minimal.

2. *Hemoglobinuria and Blackwater Fever.* The presence of hemoglobinuria in 3 and probably 4 of our cases calls for further discussion.

In every malarial chill there is, of course, more or less destruction of red blood cells; indeed, fever is not believed to be produced unless at least 1 in 10,000 red cells are parasitized.¹² The cases presented in this paper are those in which the amount of pigment so liberated into the blood stream has been sufficient to exceed the reserve capacity of the liver to excrete

it as bile, with resulting bilirubinemia and jaundice.

Dameshek and Schwartz¹ have shown that hemoglobinuria will occur when a hemolytic reaction attains sufficient intensity. In their experiments they prepared in rabbits a hemolysin against guinea-pig red cells. When a small amount of this substance was injected into guinea-pigs, a subacute hemolytic anemia ensued; a larger amount resulted in acute hemolytic anemia, and a still larger quantity produced acute fulminating anemia with hemoglobinuria.

That their findings apply equally to malarial hemolysis is attested by the fact that of the 4 patients in our series who had the most intense hemolysis as gauged by the height of the serum bilirubin, 3 showed hemoglobinuria and the 4th probably had it; and none of the others did.

The renal threshold for hemoglobin is rather high; Tonfick¹¹ observed that the plasma must be quite pink before hemoglobinuria can occur, and Pierce⁵ has calculated that in man the blood must contain at any given time 0.06 gm. of free hemoglobin per kilo of body weight before that substance can appear in the urine. In a 70 kilo man with a plasma volume of 3.8 liters, this would represent a plasma level of approximately 110 mg. per 100 cc.

It is unfortunate that we have no studies of plasma hemoglobin in any of our cases; but it is reasonable to assume that those patients who showed evidence of acute hemolysis only slightly less severe than our 3 patients with hemoglobinuria would have shown definite hemoglobinemia. The studies of Voigt and Voigt¹³ indicate that hemoglobinemia is by no means rare in uncomplicated falciparum malaria. They determined the plasma hemoglobin in 17 patients during an attack of that disease, and found that 9 showed none, 4 traces, and 4 levels varying from 22 to 60 mg. per 100 cc.; 25 normal control subjects never exceeded a level of 19 mg. per 100 cc. In 4 of 17 cases of ordinary falciparum malaria, then, significant hemoglobinemia was pres-

ent—a figure which suggests that the condition is vastly more common in falciparum malaria than is clinical jaundice, and therefore that less hemolysis is required to produce definite elevation of serum hemoglobin than to cause jaundice.

Hemoglobinemia reflects the functional failure of the reticulo-endothelial system to keep the plasma clear of hemoglobin by converting it promptly to hemobilirubin. Hemoglobinuria represents a far more advanced degree of functional impairment, inasmuch as the plasma hemoglobin cannot be kept below the renal threshold. The latter condition is far less common; it is also far more serious, for it at once jeopardizes renal function, whereas hemoglobinemia *per se* appears to entail no particular deleterious consequences.

The continuity of our cases, ranging as they do from minimal hyperbilirubinemia to marked icterus with hemoglobinuria, conveys no hint that metabolic idiosyncrasy is playing any rôle; and the conclusion is drawn that *hemoglobinuria will appear in malarial jaundice when the usual hemolytic mechanism present in the disease becomes so intense and acute that the reticulo-endothelial system is unable to keep the plasma hemoglobin level below the renal threshold.*

None of our cases merits the appellation of blackwater fever; yet, although we have not seen that condition, it is believed that some light may be shed upon its etiology by a consideration of the cases here reviewed. According to Stitt,⁹ its pathognomonic features are, explosive, severe hemolytic anemia and jaundice with massive hemoglobinuria accompanying an unusually prostrating chill in a patient with malaria. It is easy to understand how clinicians, observing the striking picture of prostration, the massive hemoglobinuria, and the greatly enhanced mortality of the condition, should suppose that they were dealing with some etiologic factor in addition to that of an egregiously severe malarial hemolysis.

From what has already been said, how-

ever, it is clear that hemoglobinuria may be expected in severe malarial jaundice. Fairley and Bromfield² found that the average maximal indirect van den Bergh reaction on the sera of 20 patients with severe blackwater fever was 10.7 mg. per 100 cc.—a figure approaching 5 times the average maximal of our cases, though but slightly higher than the 2 highest readings of our series (9 mg. and 8.5 mg.), both of which occurred in patients in whom hemoglobinuria was present. As regards the high mortality rate of blackwater fever, Stitt states¹⁰ that “the prognosis is especially dependent upon the amount of red corpuscles destroyed and whether the kidneys continue to function.” Peculiarly significant, therefore, is the fact that the 2 of our patients (Cases 20 and 24) who had the most severe hemolytic reaction, and whose urines were among the 3 which definitely contained hemoglobin, developed a severe acute nitrogen retention (B.U.N. 105 and 113, respectively), and, in the case of the former, evidence of serious derangement of renal function (impaired urinary concentration).

It is suggested that our cases exhibit a series of instances of malarial hemolysis ranging from the least amount required to produce icterus to a severe jaundice with hemoglobinuria which is just short of true blackwater fever; that a somewhat more hyperacute malarial hemolysis than we have seen would exemplify the blackwater fever syndrome; and that the appearance of hemoglobinuria, with its threat to renal function in an acutely ill febrile patient, though implying an abrupt enhancement of the risk to health and life, does not necessarily indicate any etiologic hiatus nor imply any causative factor other than unusually severe malarial hemolysis.

Summary and Conclusions. Analysis is made of 24 cases of jaundice caused by malaria, comprising 0.24% of 8831 cases of malaria treated in the 20th General Hospital over a 28 month period.

Malarial jaundice is a distinct clinical entity characterized by the abrupt onset

of painless icterus in a febrile patient suffering with malaria. It is of short duration, averaging 7 days in our series. Enlargement of the liver and bilirubinuria are common; acholic stools probably do not occur. It is 4 times more common in *falciparum* than in *vivax* malaria.

It is primarily a hemolytic icterus, but hepatic injury secondary to hemolysis and fever contributes to the production of jaundice. There is no evidence that quinine or atabrine have any part in its pathogenesis.

Hemoglobinuria is the rule in exceptionally severe malarial jaundice. Because of its threat to renal function it is a complication to be dreaded. The mechanism of its production is discussed. Cerebral malaria probably accounted for all 3 fatalities in our series, but its incidence appears to be unrelated to the presence of jaundice. Uncomplicated malarial jaundice is benign.

The relationship of malarial jaundice to blackwater fever is discussed.

ADDENDUM. Since the completion of this paper, Major T. E. Machella has informed me that in the earliest days of this hospital he cared for 3 patients with unquestionable blackwater fever. It is a great misfortune that their records are no longer available, as they would have completed the continuity of a series of patients with malarial jaundice seen in this hospital ranging from minimal icterus up to and including the blackwater fever syndrome.

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PURPURA AS A COMPLICATION OF MALARIA

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A COMPLICATION of a disease, although it may occur only once in 1000 cases, becomes important when millions of men are exposed to this disease, especially if the complication is a serious one and the danger to life is great. This is true of hemorrhage or purpura which occurs during the course of malaria, or as a result of drug therapy in malaria.

Purpura as a complication of malaria has been noted in the past by various investigators, although reports on this subject have been infrequent during the past 2 decades. Paiseau and Lemaire¹⁰ observed hemorrhagic manifestations between the 1st week and the 2nd month after primary infection with plasmodia. "They have met with cases of simple purpura, manifested in fine petechiæ all over the body but particularly abundant on the legs, and characterized by a complete absence of pyrexia; of simple purpura shown in extensive ecchymoses and associated with ordinary 'pernicious' symptoms; of hemorrhagic or hemophilic purpura, where the cutaneous ecchymoses are quite overshadowed by hemorrhages from the mucosæ and particularly epistaxis, and the peripheral blood contains many immature elements and basophil multinuclears; of hemoglobinuric purpura, in which, besides petechiæ and ecchymoses and epistaxis and bleeding from the gums, hemoglobinuria was a conspicuous symptom only 4 days after the initial paroxysm of a primary infection; and of pernicious subicteric anemia, where within 23 days of the initial (and probably primary) infection, extremely severe epistaxis, concurrent with ecchymoses and petechiæ, were prominent symptoms of a progressive anemia that ended fatally within a month."

Christin³ reported a case of purpura

involving both skin and mucous membranes occurring in a chronic malarious subject who had marked anemia and leukopenia. Lorando, Chaniotis and Chouretis⁵ recorded a case of tertian malaria in which each febrile attack was followed by an urticarial eruption accompanied by the appearance of purpuric patches. Mickaniewski and Hai⁸ described 5 cases of purpura in Indo-China occurring in 3 young men, a woman, and a girl of 15, all of whom were suffering from chronic subtertian malaria of several years standing. None of them had taken quinine.

Purpura occurring as a result of the use of antimalarial agents, particularly quinine, has also been recorded. Thus, Deeks⁴ referred to hemorrhages into the mucous membranes and extensive purpura as a complication of malaria treated with quinine. Vialatte and Remontet¹⁷ reported a case of purpura due to quinine used as a suppressive antimalarial agent. Regendanz¹² noted the occurrence of petechiæ when quinine was used for malaria prophylaxis in troops. Barnes¹ observed purpura in a Chinaman treated with quinine. Maritschek and Markowicz⁷ reported a case of recurring purpura due to quinine. Manson-Bahr,⁶ Quick,¹¹ Christian,² Sollmann,¹³ and Solis-Cohen,¹⁴ among other authorities, have commented upon the fact that quinine therapy may result in purpuric manifestations. Nocht and Mayer⁹ stated: "It is not yet known for certain to what extent hypersensitivity to quinine and in particular the hemorrhagic diathesis frequently occurring after quinine is due to concurrent malaria infection. But it seems as if in some cases the tendency to hemorrhage after the administration of quinine can be induced by malaria alone without a previous misuse of quinine

being in any way responsible. On the other hand, severe, even fatal, hemorrhages after quinine have been observed for which malaria was in no way responsible."

Data. The records of 10,000 consecutive cases of malaria in Gorgas Hospital were checked for hemorrhagic complications. Of these 10,000 patients, 6000 were treated with quinine, 2500 with atabrine. Ten patients (0.1% of the total) showed evidence of purpura. In only 1 instance (Case A) (0.01% of 10,000 cases) was purpura present before treatment. In the other 9 cases purpura occurred after treatment with quinine. The incidence of purpura in individuals treated with quinine, used alone or with atabrine, was 9 in 7500 (0.12%). There was not a single instance of purpura in a patient given atabrine alone.

In 9 cases the purpura occurred from 1 to 8 days following the onset of clinical symptoms of malaria. In each instance, however, the purpura followed the institution of quinine therapy. Six of these patients had been treated for malaria with quinine prior to the current infection or had been on "suppressive" or "prophylactic" quinine therapy. Of these, 1 (Case J), who had previously been on suppressive quinine therapy without symptoms, developed subconjunctival hemorrhages when treated with quinine for his first attack of malaria in March 1942. In April 1942, he had his second attack of malaria, which was treated with atabrine without the development of hemorrhagic manifestations. At the onset of the last attack of malaria, on May 20, 1942, the patient took 0.67 gm. of quinine and noted ringing in his ears and dizziness. On admission to the hospital, May 24, 1942, bilateral conjunctival hemorrhages were noted. The other 5 patients had no history of previous purpuric manifestations.

With but 1 exception, all of the patients were young, adult, white males who were members of the U. S. Army. The 1 exception was a middle-aged, white American male (Case I). In no instance was any history of an allergic background elicited on the part of a patient or a patient's family.

The clinical and laboratory data of the 9 patients (Cases B and J) who developed purpura after receiving quinine are sum-

marized in Table 1. The determination of the severity of the illness was based upon the clinical course of the primary illness, namely malaria, and the severity and extent of the purpura. In 1 patient (Case E) there was the complication of oliguria. Terminal pulmonary edema was present in both patients who died.

The treatment of the 9 patients was essentially similar. In all, quinine was discontinued and atabrine substituted. Six received infusions of saline and glucose; 3, whole blood transfusions; and 1, blood plasma. Oxygen was administered to 3 and "alkalinization" was attempted in 3. Anti-hemorrhagic agents such as calcium gluconate and vitamin K were employed in the treatment of 2 patients.

Illustrative Case Reports. **CASE A.** A 33 year old, white soldier was admitted complaining of dull headache, weakness, generalized body aches, bloody vomitus, and diarrhea of 4 days' duration. The temperature was 104.2° F., pulse was rapid, weak, and thready (rate about 200 per minute), blood pressure was 95 mm. of mercury systolic and 65 mm. of mercury diastolic. Subconjunctival hemorrhage, icteric tinge to scleras, herpes labialis, small hemorrhagic areas scattered throughout the buccal mucous membrane and soft palate, pharyngitis, palpable tender spleen, and a palpable tender liver were noted on physical examination. The bleeding time was 3 minutes; the coagulation time, 3½ minutes. *Plasmodium falciparum* was found in the blood film. The patient was placed upon quinine therapy despite the presence of purpura and was given supportive treatment. In all, he received 34 gm. of quinine in a 16 day period, and his response to the therapy was excellent. He improved markedly within 24 hours and all evidences of malaria and of the purpura disappeared within 5 days of the beginning of therapy. This, then, is an example of purpura occurring apparently as a part of the malarial picture before any antimalarial drug was given and responding excellently to quinine therapy.

CASE F. A 31 year old, white Army officer was admitted complaining of chills, fever, general malaise, slight cough, weakness, and headache of 2 days' duration. The patient had a temperature of 104.4° F., hot, dry skin, conjunctivitis, injected pharynx, tachycardia, and a palpable spleen. The

TABLE 1.—DATA ON 9 CASES

Case	Type of malaria	History taking quinine previously	Total quinine taken before onset of purpura (gm.)	Time between first dose and onset of purpura (hrs.)	Purpuric manifestations	Platelet count (thous. per c.mm.)	At time of purpura			Duration of purpura (days)	Severity of illness
							Bleed. time (min.)	Cong. time (min.)	R.B.C. count (mill.)		
B	<i>P. vivax</i>	+	5 0	29	Subconjunctival hemorrhage	102	Not done		3 93	1	Mild
C	<i>P. vivax</i>	+	2 67	Less than 12	Petechiae over anterior aspects of both legs, upper extremities and thorax	24	2½	4½	3 75	3	Mod.
D	<i>P. vivax</i>	+	2 67	Noted in 1st urine spec. follow. drug	Erythrocytes in urine; bloody sputum	36	2½	4	3 65	1	Died
E	<i>P. falciparum</i>	+	2 0	16½	Bloody vomitus; tarry stools; blood in urine; bloody sputum	49	Not done		2 25	5	Died
F	<i>P. falciparum</i>	—	4 0	14½	Macular rash over entire body; bloody vomitus	212	Not done		3.70	1	Mod.
G	<i>P. falciparum</i>	—	5 0	28	Blood in stool and urine	Not done	Not done		5 00	4	Severe
H	<i>P. vivax</i>	—	3 33	4½	Gross hemorrhage from bowel and petechiae of skin of left flank, axillae and buccal mucous membrane	129	2	5	2 17	5	Severe
I	<i>P. vivax</i>	+	4 0	Approx. 24	Petechiae on skin of arms, legs, abdomen	160	"Normal"		3.85	5	Mod.
J	<i>P. vivax</i>	+	0 67	Not known*	Bilateral subconjunctival hemorrhage	216	3	2	4 44	4	Mild

* Noted 4 days later.

laboratory findings were as follows: leukocytes, 13,450 per c.mm. of blood; erythrocytes, 3,700,000 per c.mm. of blood; hemoglobin, 80%; blood film positive for *P. falciparum*. Quinine therapy was instituted, the patient receiving 2 gm. orally at 1 A.M., 1 gm. at 7 A.M., and 1 gm. at 1 P.M. At 3:45 P.M., 14 $\frac{1}{4}$ hours after the institution of the quinine therapy, the patient vomited 2 ounces of bloody material, passed urine containing erythrocytes, and a macular rash appeared over the entire body; areas of "redness" were noted on the right cheek. He also had a shaking chill, tinnitus aurium, and deafness. The platelet count at this time was 212,000 per c.mm. of blood. Quinine was discontinued and the patient was placed upon atabrine therapy, the hemorrhagic manifestations ceasing in 1 day and all symptoms clearing up within 4 days. This is a case of non-thrombocytopenic purpura occurring in a patient with malaria following the institution of quinine therapy.

CASE C. A 22 year old, white soldier was admitted on Jan. 4, 1943, with fever, chills, headache, dyspnea on exertion, abdominal cramps, and loss of appetite. He also complained of a dry, hacking cough and night sweats of 3 months duration. In June 1942, while the patient was stationed at a jungle position, he had symptoms of malaria and treated himself with quinine, taking 3 gm. daily for 7 days without any untoward effects. The patient had 2 attacks of malaria diagnosed by blood film examination in September and in November 1942. From Dec. 14, 1942, to Jan. 2, 1943, the patient took 0.6 gm. of quinine daily without any signs of sensitivity. On Jan. 3, 1943, he took 2.6 gm. of quinine in a single dose because he believed that his symptoms were due to malaria. On Jan. 4, 1943, when the patient entered the hospital, petechiæ were found scattered in the skin over the malleoli and anterior aspects of both lower extremities, the thorax, and the upper extremities. The tonsillar pillars and pharynx were injected and the postcervical lymph nodes were enlarged. There were coarse râles in the apices of both lungs, epigastric tenderness, and the spleen was palpable. Roentgen ray examination of the chest was negative. *P. vivax* was found in the blood film. At the time of admission his platelet count was 24,000 per c.mm. of blood; erythrocytes, 4,650,000 per c.mm. of blood; and leuko-

cytes, 7600 per c.mm. of blood, with a normal differential. The bleeding and coagulation times were normal. Quinine was discontinued, a course of atabrine treatment was instituted; 2 days after completion of atabrine therapy a course of plasmochin was started. The petechiæ had faded by the 3rd day of atabrine treatment. At the end of the week, the platelet count was 213,000 per c.mm. of blood and the patient made an uneventful recovery. This is a case of thrombocytopenic purpura secondary to the use of quinine in the treatment of malaria.

CASE E. A white soldier, age 22, was admitted on Jan. 29, 1942, with the chief complaints of muscular aching, malaise, frontal headache, shaking chill, fever, and repeated vomiting. In September 1942, the patient had been admitted to the hospital with a fever of undetermined origin which responded quickly to quinine therapy. Just 1 week prior to the terminal admission the patient had been discharged from the hospital after being treated for estivo-autumnal malaria. On his third and final admission the scleras were slightly icteric, the pharynx showed a diffuse hyperemia, and the spleen was palpable. No plasmodia were found on examination of the blood during this admission. He was given 2 gm. of quinine at midnight. The following day the patient passed bloody urine, vomited blood, and had tarry stools. His red cell count dropped to 2,950,000 per c.mm. of blood, hemoglobin to 50% (Sahli method). The white cell count rose to 19,000 per c.mm. of blood, with a differential of 80% polymorphonuclears and 20% lymphocytes. The platelet count was 49,000 per c.mm. of blood. A tourniquet test applied to the arm was negative. Quinine was discontinued, the patient was placed on atabrine routine, given infusions of saline with 10% glucose, 3 blood transfusions, and oxygen therapy. By the 6th day of his illness he developed severe oliguria, marked pulmonary edema, and expectorated bloody sputum. The patient died on Feb. 3, 1942. Autopsy revealed pulmonary edema, hemorrhagic nephritis, and perirenal hemorrhage. This is a case of thrombocytopenia with hemorrhage into the gastro-intestinal and urinary tracts following the use of quinine.

Comment. Although only 0.1% of the cases of malaria treated at Gorgas Hospi-

tal manifested hemorrhagic tendencies, these were of grave import, necessitating the placing of 4 of the 10 patients with purpura on the seriously ill list.

That this condition may be an allergic manifestation and not a simple toxic one is suggested by the fact that one-half of the patients who developed purpuric manifestations had received quinine previously without developing any untoward symptoms, the wide variations in the amount of quinine taken before the development of purpura, and the fact that the hemorrhagic picture in the patient who had not been treated and in those who were treated with quinine was similar.

Tuft¹⁵ stated: "Drug allergy or idiosyncrasy refers to a condition of hypersensitiveness or allergy in which the administration of a medicinal substance in a quantity non-toxic for the average individual is followed by an unusual but characteristic reaction. This type of reaction is distinguished from that due to the toxic action of the drug by the fact that the latter produces symptoms which are an exaggeration of its physiologic action." The total amount of quinine which the patients received before developing purpura was never more than 5 gm. in divided doses and most times less. Thousands of patients took more quinine without developing purpura. This purpura, then, was not due simply to the toxic effect of quinine. Vaughan¹⁶ stated that symptoms of drug allergy "may follow the first recognized or traceable contact with the drug. Usually there is history of previous administration of longer or shorter duration, often with an intervening period without the drug." This fits in very well with the history of our patients, many of whom received quinine over a prolonged period of time before the onset of purpura.

For these reasons it appears that purpuras arising in patients treated with quinine are expressions of allergy. The fact that in the absence of malaria some of the patients took quinine without any reaction, but developed the hemorrhages

in the presence of malaria, may indicate that malaria itself can act as a sensitizing agent for the production of an allergy to quinine. Indeed, the appearance of purpura in cases of malaria in the absence of any antimalarial drug suggests that the plasmodium or its toxins may be capable of acting as an allergin. Conversely, it is conceivable that quinine may act as a sensitizer to the malaria allergin which may produce purpura. These are mere speculations which await investigation.

The occurrence of purpura in white Americans only in Gorgas Hospital, where a large proportion of the patients with malaria are native Panamanians and British West Indian Negroes, is puzzling. While some of the skin manifestations might have been overlooked in these groups of patients, certainly the severe visceral manifestations could not have escaped notice. Part of the answer may be that these native groups have not been exposed to the evil of sensitization by repeated dosage with quinine used in a so-called "suppressive" or "prophylactic" manner. It is the impression of some members of the hospital staff that allergic manifestations as a whole are less frequent in the native population groups than in the Americans.

Although quinine may be instrumental in causing a hemorrhagic diathesis, it may be given in untreated malaria complicated by purpura with good results, but by far the wiser course would be to use atabrine which, in over 2500 cases of malaria, was not associated with purpura.

The 2 deaths bring out an important point. Persons sensitive to antimalarial drugs run an additional hazard of death in malarious districts. It is the policy of the Medical Examining Board of Gorgas Hospital to declare such individuals unfit for tropical duty.

Summary. 1. In 10,000 consecutive cases of malaria treated at Gorgas Hospital there were 10 cases (0.1%) complicated by purpura. There was 1 case of purpura in a patient with untreated

malaria, but in 9 cases the purpura followed the use of quinine.

2. The incidence of purpura in cases treated with quinine was 9:7500 (0.12 %).

3. There were no instances of purpura following the use of atabrine in over 2500 cases of malaria.

4. Purpura occurred as frequently in cases of tertian malaria as in estivo-autumnal malaria.

5. The purpuras were evenly divided between thrombocytopenic and non-thrombocytopenic varieties.

6. The regions of the body affected were the gastro-intestinal tract, genito-urinary tract, skin, conjunctiva, and respiratory tract, in that order.

7. The withdrawal of quinine was of value in those cases in which the purpura was due to quinine, but in the patient without previous antimalarial therapy quinine was used successfully despite the presence of purpura.

8. Other measures used in the treatment of malaria patients with purpura were the institution of an atabrine routine, intravenous infusions of saline and glucose, blood transfusions, plasma transfusions, alkalization of the patients, oxygen therapy, and the use of calcium gluconate and vitamin K.

9. The purpuric manifestations persisted for from 1 to 5 days after the institution of measures to control them. The complication was a serious one, necessitating the placing of 40 % of the affected patients on the seriously ill list. Two patients died.

10. All patients affected were white American males, mostly belonging to the younger age group. No purpura occurred in the native population which made up a large number of the malarial patients.

11. We suggest that purpura due to quinine may be an allergic phenomenon and not simply a toxic manifestation.

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THE BLOOD PROTEIN TYROSINE REACTION IN MALARIA, ACUTE EPIDEMIC HEPATITIS, AND CERTAIN OTHER DISEASES

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In the past, several seroflocculation tests have been described for the diagnosis of malaria.^{1,3,6} Such tests, if proved reliable, would be a useful diagnostic aid, particularly in patients who have clinical evidences of malaria, but repeatedly negative thick blood films. One of the most easily performed of the seroflocculation tests is the protein tyrosine reaction described by Proske and Watson⁶ in 1939. The purpose of this investigation was to evaluate the blood protein tyrosine reaction in the diagnosis of malaria and certain other diseases, including acute epidemic hepatitis (acute infectious hepatitis, acute catarrhal jaundice, acute infectious jaundice, etc.).

Proske and Watson have reviewed the literature on seroflocculation tests in malaria and other diseases, and the reader is referred to their article for details. Although differences of opinion exist concerning the significance of these tests, and various theories have been advanced to explain the mechanism of abnormal flocculation, available evidence indicates that a positive reaction may occur in any disease in which there is a disturbance of serum globulin. Proske and Watson reported that the protein tyrosine test was elevated in patients with malaria. In normal persons the protein tyrosine test varied between 50 and 80 (arbitrary units), whereas in patients with malaria the range was 105 to 280 or higher. They found blood protein tyrosine elevated in 97% of cases of clinical malaria, whereas positive thick blood smears were obtained in only 82% of the same cases. A rise in blood protein tyrosine before the appearance of

clinical symptoms in 1 patient indicated that the test might be useful in predicting a relapse of malaria.

Technique. According to Proske and Watson, the protein tyrosine reaction is based on the fact that "proteins possess a chromogenic property which can be measured quantitatively against the color produced by pure tyrosine in the presence of a phenol reagent. This chromogenic value is constant for a given protein and the intensity of the color produced can be used as a measure of the amount of the protein examined." The color is developed by means of a phenol molybdate-tungstate reagent, which presumably reacts with the phenol group of tyrosine in the protein. The precipitated protein is thought to be euglobulin. We have used the method described by Proske and Watson.

Reagents. 1. Sodium sulfate solution (14%): dissolve 70 gm. of c.p. anhydrous sodium sulfate in 300 ml. of freshly distilled water and make up to 500 ml. at 37° C. This solution is stored in an incubator at 37° C. and keeps indefinitely.

2. Sodium hydroxide solution (5 N): dilute saturated, carbonate-free sodium hydroxide solution to 20% with distilled water.

3. Tyrosine standard solution: dissolve 200 mg. of tyrosine (Pfanstiehl) in 1000 ml. of 0.1 N hydrochloric acid, which makes a solution containing 1 mg. of tyrosine per 5 ml. of 0.1 N hydrochloric acid. This stock solution is kept in a refrigerator.

4. Phenol reagent of Folin and Ciocalteu:² into a 1500 ml. Florence flask introduce 100 gm. of sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) 25 gm. of sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$), 700 ml. of distilled water, 50 ml. of 85% phosphoric acid (H_3PO_4) and 100 ml. of concentrated hydrochloric acid. Reflux gently for 10 hours. Add 150 gm. of lithium

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sulfate (Li_2SO_4), 50 ml. of distilled water and a few drops of bromine water. Boil the mixture for 15 minutes without the condenser to remove excess bromine. Cool, dilute to 1000 ml., and filter. Freshly prepared reagent should have no greenish tinge, but, if this develops with age, the solution

the tube, and place in an incubator at 37°C . for at least 3 hours. As a matter of convenience the mixture may be left in the incubator overnight. At the end of incubation centrifuge the mixture at about 1500 r.p.m. for 10 minutes. Completely remove the supernatant fluid with a pipette and

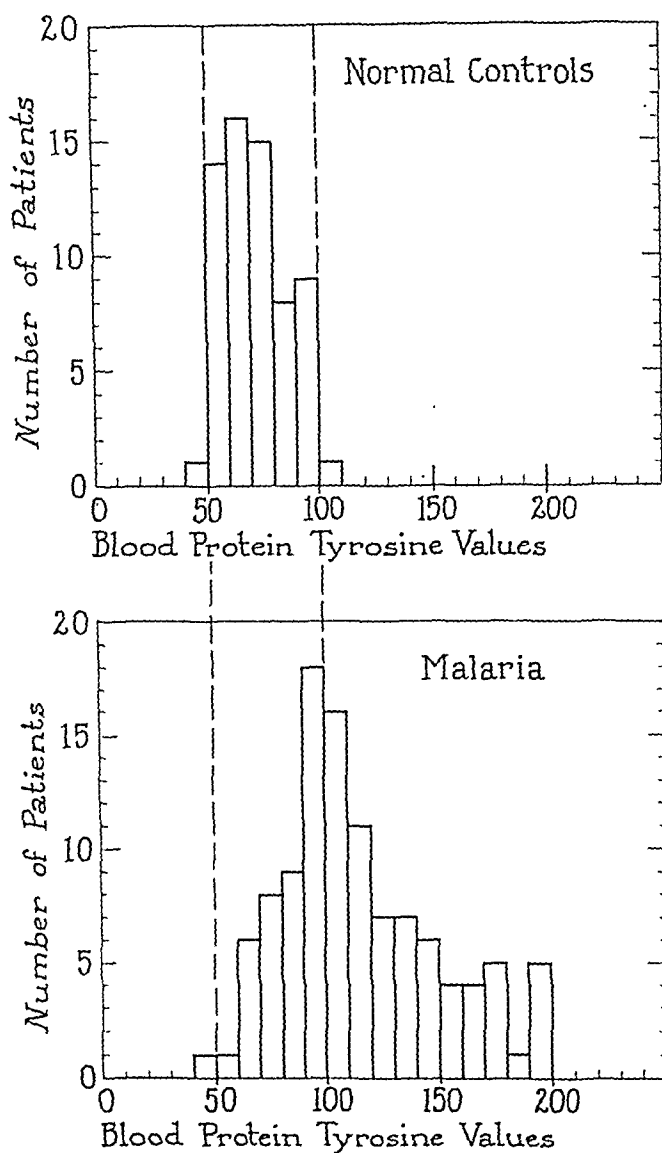


CHART 1.—Comparison of blood protein tyrosine of malaria patients with normal individuals. The 2 broken vertical lines show the normal variation of the test. Although the average value for patients with malaria was increased, nevertheless 40% of the values were in the normal range.

can be made usable by addition of a few drops of bromine water, boiling for a few minutes to remove excess bromine, and stopping when the solution is golden yellow.

Procedure. Measure 3 ml. of 14% sodium sulfate solution into a small test-tube and add 0.1 ml. of unheated, clear, non-hemolyzed serum. Mix by inversion, stopper

wash the precipitate twice with fresh sodium sulfate solution by centrifugation. Dissolve the washed precipitate in 1.75 ml. of distilled water and add 0.1 ml. of 5 N sodium hydroxide.

The standard is prepared by placing 2 ml. of tyrosine solution, 5 ml. of distilled water and 1 ml. of 5 N sodium hydroxide in a

20 ml. graduated tube. Heat unknown and standard in boiling water for 10 minutes and allow to cool. Add to the unknown 0.15 ml., and to the standard 1.5 ml. of phenol reagent, and make up the standard to the 20 ml. mark with distilled water. The unknown may be compared with the standard in a colorimeter or with a series of substandards prepared by diluting the standard with suitable amounts of distilled water.

TABLE 1.—VALUES OBTAINED AT VARIOUS TIME INTERVALS

Time interval	No. cases	Mean protein tyrosine (arbitrary units) with the standard error
1st 2 weeks	58	125± 4 99
2nd 2 weeks	22	113± 3 86
3rd 2 weeks	25	90± 2 70
Last 2 months	27	64± 3 27

The results were expressed in arbitrary units, rather than in mg. because the test measures a protein fraction or fractions, the exact nature of which is unknown; namely, the fraction precipitated by 14% sodium sulfate solution. The results have been calculated according to the following formula: $\frac{\text{Standard}}{\text{Unknown}} \times 100 = \text{protein tyrosine}$. This procedure is sufficient for clinical purposes, where interest is in a deviation from normal and not necessarily in absolute values.

Results. In order to determine normal variations of the test 60 determinations of blood protein tyrosine were made on 33 healthy young men, 20 to 35 years of age (Chart 1). The average blood protein tyrosine value was 70. Because values varied from 50 to 100, these limits were chosen as the normal range, and any test above 100 was considered abnormal. Duplicate tests on the same sample of serum checked within 5%, although tests done on the same person sometimes varied as much as 20 units on different days, but always within normal limits. Determinations were made before and after meals, at various times during the day, after exercise, and following exposure to cold, but these procedures had no effect upon the test. In order to study the effect of elevation of temperature on the test, determinations were made on 2 patients with chronic gonococcal urethritis,

who were being treated in the fever cabinet. No change was observed during fever therapy, but 1 patient became slightly jaundiced (icterus index 15) 4 days later, and protein tyrosine rose from 83 to 108, returning to normal after 7 days.

Malaria. Protein tyrosine determinations were made on 110 patients with clinical evidences of *Plasmodium vivax* malaria (benign tertian) and positive

blood smears. No cases with *P. falciparum* or *P. malariae* infections were studied. All patients were young, white men 18 to 30 years old, who previous to military service in the tropics were in excellent health and had not had malaria. During the course of their duties they were in a highly malarious district under combat conditions for a period of about 4 months before they were evacuated to a temperate region in which there was no malaria. The first cases of malaria occurred at the end of 2 weeks in the tropics, and the peak incidence was reached at the end of 3 months, after which the disease persisted for as long as the troops remained in that area. They were treated with quinine and atabrine upon appearance of symptoms, and after the first few weeks most of them received suppressive treatment consisting of atabrine 0.1 gm., twice a day every 3rd day. In most cases suppressive treatment was stopped soon after evacuation. Protein tyrosine determinations were started 3 months after the patients had moved to a temperate climate. As soon as plasmodia were discovered in thick blood films, blood was drawn for the first protein tyrosine determination; other determinations were made at intervals of 1 week. Three determinations were made in most cases.

Chart 1 shows the results which are plotted with the control series for purposes of comparison. The average value was

125, which is definitely abnormal, but a study of the distribution of the readings showed that 40% of them were in the normal range. This means that a significant proportion of patients who were tested when malaria parasites were demonstrable in the blood stream had a negative tyrosine reaction.

Malarial Relapse. The relapse rate during the period of investigation was 35%. That is to say, of the 110 patients, 38 returned to the hospital within 3 months with signs of clinical malaria and positive blood smears. This fact raised the question of the value of the test in predicting relapse. Did patients with an

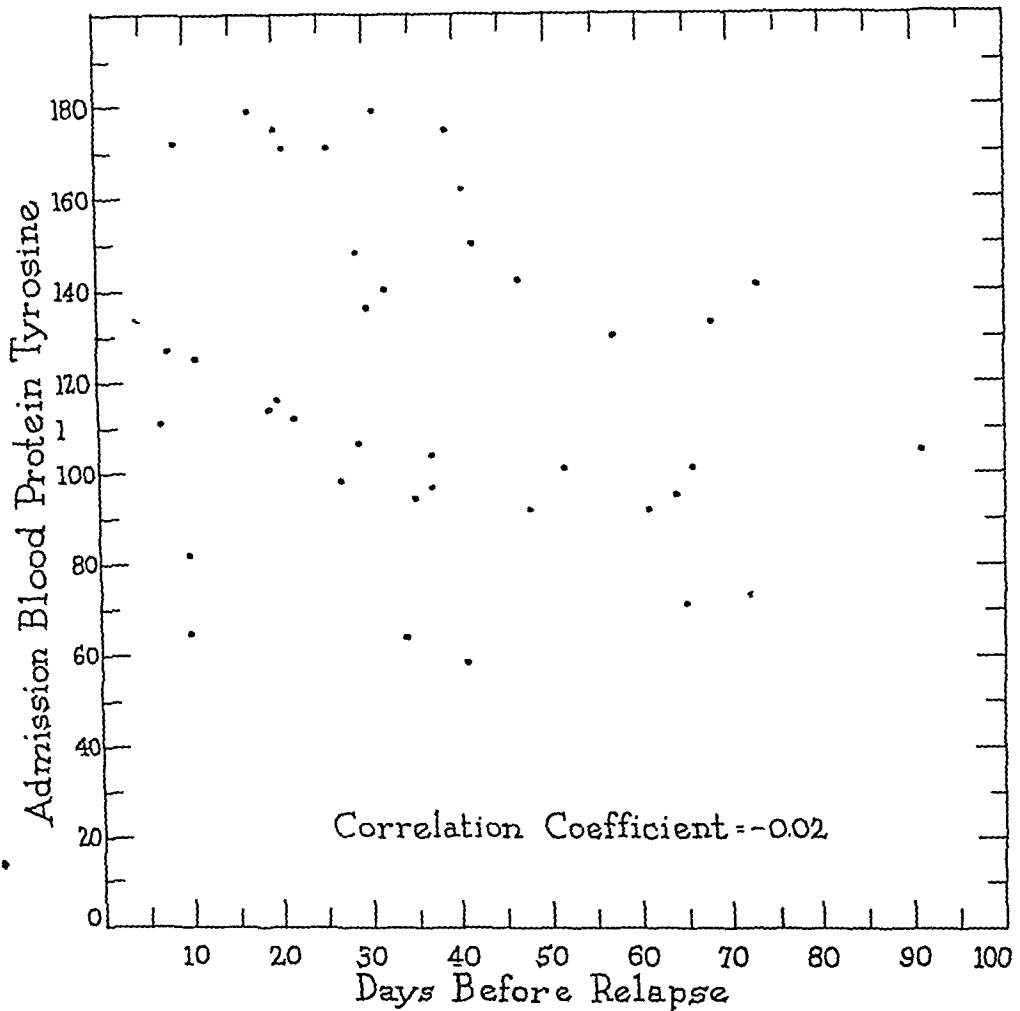


CHART 2.—Scatter diagram of the blood protein tyrosine of 110 patients on their first hospital admission, and the number of days elapsing before readmission to hospital because of a relapse. There is no significant association between the 2 sets of observations.

Comparison of results obtained early in the investigation with those obtained later (Table 1) showed that the protein tyrosine reaction gradually returned to normal in most patients. In the first 6 weeks there was a gradual fall in the mean value from 125 to 64, at which level the tests remained stationary during the rest of the experimental period; a matter of 3 months.

increased blood protein tyrosine relapse sooner than patients with normal values? Was there a tell-tale increase just before a relapse?

Chart 2 gives the answer to the first part of the question. This chart is a scatter diagram of admission protein tyrosine readings and the number of days elapsing before readmission to hospital because of a relapse. These readings are

obviously so widely scattered that there is no significant association between the 2 sets of observations. The correlation coefficient for the data is -0.02 , which indicates a lack of correlation because it approaches closely to zero. In other words, admission blood protein tyrosine values, whether high or low, were not useful in predicting relapse.

In answer to the second part of the question it may be said that only 8 patients (22.8%) of those readmitted for relapse had an increased blood protein tyrosine. The other patients had normal values on readmission. Thus it must be concluded that the blood protein tyrosine reaction is not helpful in predicting malarial relapses.

Acute Epidemic Hepatitis. A question arose, during the course of this work, as to the behavior of the protein tyrosine reaction in diseases other than malaria. Of the diseases which were studied, the only one in which the protein tyrosine content of blood was frequently elevated was acute epidemic hepatitis. Twenty-seven patients were examined in whom the diagnosis of acute epidemic hepatitis had been made on the basis of clinical findings plus an increased icterus index (Table 2). In 11 cases cephalin flocculation tests were also done, but no attempt was made to investigate liver function in detail. All were young adult soldiers, 18 to 30 years old. The causative agent of the disease in this group of patients was not discovered.

Readings were made at weekly intervals, but some of them have been omitted in order to shorten the table (Table 2). Enough have been included, however, to show the behavior of the protein tyrosine and icterus index in each case.

Elevation of blood protein tyrosine occurred sometime during the disease in all except 2 patients (Cases 9 and 12), 1 of whom had a 1+, and the other a 3+ cephalin flocculation test. There is no apparent explanation for these 2 cases.

Whenever the protein tyrosine was over 100 the cephalin flocculation reaction, in

all cases tested, was 4+. Two and 3+ cephalin flocculation tests were seen in cases with normal protein tyrosine values, and also in a few apparently healthy individuals.

Miscellaneous Diseases. Blood protein tyrosine was determined in a variety of diseases (Table 3), in most of which the protein tyrosine was not increased. However, an elevation of protein tyrosine occurred in 2 cases of enteric fever, and 1 each of lobar pneumonia, meningococcic meningitis, and acute infectious mononucleosis. With the exception of the case of lobar pneumonia, which showed a persistent and marked elevation, the increase in protein tyrosine was transient and moderate. Whether or not this increase was significant can only be determined by further study.

The patients with chronic calculous cholecystitis (pathologic diagnoses) were slightly jaundiced, but at operation no stones were found in either common or hepatic bile ducts.

Discussion. As stated earlier, the original purpose of this investigation was to determine the usefulness of the blood protein tyrosine reaction in the diagnosis of malaria. The results show that the test was not of diagnostic value in the particular group of patients studied, and under the experimental conditions which prevailed. This is clearly indicated by the fact that many patients had a normal blood protein tyrosine level at the same time that large numbers of plasmodia could be easily demonstrated in peripheral blood. It was also impossible to predict a relapse by means of the test.

Although these findings seem to conflict with those of Proske and Watson, the discrepancy is probably not as great as might appear at first sight. It is important to note that the 2 investigations were carried out on 2 different groups of patients and under different circumstances. Proske and Watson tested patients who lived in an endemic area and probably suffered from chronic malaria. In contrast, our patients had relatively acute

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TABLE 2.—DATA ON 27 CASES OF EPIDEMIC HEPATITIS

Case	Hosp. days	Date, 1943	Icterus index	Protein tyrosine (arbitrary units)	Cephalin flocc.	Liver	Last attack of malaria	Remarks
1	138	Mar. 22	144	234	...	Liver Large tender	Jan. 1943	Jaundice present 2 mos. before protein tyrosine test; May 24, discharged "cured" before another reading was made
2	85	Apr. 7	55	156	...	Large tender	Jan. 1943	Relapse of jaundice June 1
		May 1	23	99	...	Large tender		
		May 10	9	115	++++			
		May 17	7	217	++++			
		May 8	21	119	++++			
		May 31	10	166	++++			
		June 7	59	230	++++			
		June 21	23	172	++++			
		June 28	15	133	++++			
		July 5	15	75	++++			
		July 26	5	250	...	Large tender	Dec. 1942	June 9, discharged "cured"
3	80	Apr. 19	73	142	...	Large tender	Nov. 1942	
		May 17	10	105	...	Large tender		
		June 7	9	135	...	Large tender		
4	70	Apr. 23	162	83	+	Large tender		
		May 3	109	82	+	Large tender		
		June 14	25	103	+	Large tender		
		June 22	14	88	+	Large tender		
		June 28	10	69	+	Large tender		
		July 12	..	77	+	Large tender		
5	68	June 3	16	99	...	Normal	None	Jaundice in July, Aug., Dec. 1942; April 1943
		June 21	18	104	++++	Normal	None	
		June 28	18	80	++++	Normal	None	
		July 12	21	85	++++	Normal	None	
6	51	June 10	48	100	++	Normal	None	
		June 21	80	72	++	Normal	None	
		July 5	40	75	++	Normal	None	
		Aug. 1	8	97	++	Normal	None	
7	50	June 7	19	120	++++	Large tender	None	
		June 24	7	110	++++	Large tender	None	
		July 5	10	41	++++	Large tender	None	
		July 28	..	62	++++	Large tender	None	
		Aug. 2	3	133	++++	Large tender	None	
8	48	May 21	6	175	++++	Large tender	None	
		June 28	36	159	++++	Large tender	None	
		July 5	14	128	++++	Large tender	None	
		July 26	10	67	+++	Large tender	None	
9	35	June 14	24	83	+++	Large tender	None	
		June 21	27	56	++	Large tender	None	
		July 5	10	144	++	Large tender	None	
		Apr. 7	44	102	...	Large tender	None	
		May 5	17	98	...	Large tender	None	
		May 10	10	115	...	Large tender	None	
		May 3	50	90	...	Large tender	None	
		May 10	25	71	+	Large tender	None	
		May 17	21	88	+	Large tender	None	
		June 28	32	69	+	Large tender	None	
		July 3	96	81	+	Large tender	None	
		July 5	136	56	+	Large tender	None	
		July 12	90	112	...	Large tender	None	
		July 26	8	68	...	Large tender	None	
		Mar. 7	43	108	...	Large tender	None	
13	30	June 11	56	58	+	Large tender	None	
		June 21	15	125	++++	Large tender	None	
		July 5	8	121	++++	Large tender	None	
		May 22	60	94	...	Large tender	None	
14	28	July 9	130	64	...	Large tender	None	
		July 13	40	121	...	Large tender	None	
		July 26	10	127	...	Large tender	None	
		July 27	9	126	...	Large tender	None	
		June 21	10	108	...	Large tender	None	
		Apr. 20	4	53	...	Large tender	None	
15	28	May 17	28	64	...	Large tender	None	
		May 24	16	95	...	Large tender	None	
		May 27	11	68	...	Large tender	None	
		May 31	20	106	...	Large tender	None	
		May 17	11	94	...	Large tender	None	
		May 22	20	77	...	Large tender	None	
		May 27	40	75	...	Large tender	None	
		May 5	..	104	...	Large tender	None	
		May 10	60	76	...	Large tender	None	
		May 17	48	132	...	Large tender	None	
		May 24	16	100	...	Large tender	None	
		May 27	11	116	...	Large tender	None	
		May 31	20	115	...	Large tender	None	
		May 17	40	125	...	Large tender	None	
		May 5	Large tender	None	
		May 10	60	Large tender	None	
		May 17	48	Large tender	None	
		May 22	30	Large tender	None	
		May 27	12	Large tender	None	
		May 31	10	Large tender	None	
		May 17	40	Large tender	None	
		May 24	10	Large tender	None	
		May 27	7	Large tender	None	
		May 31	20	Large tender	None	
		May 17	4	Large tender	None	
		May 24	4	Large tender	None	
		May 27	4	Large tender	None	
		May 31	4	Large tender	None	
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		May 27	4	Large tender	None	
		May 31	4	Large tender	None	
		May 17	4	Large tender	None	
		May 24	4	Large tender	None	
		May 27	4	Large tender	None	
		May 31	4	Large tender	None	
		May 17	4	Large tender	None	
		May 24	4	Large tender	None	
		May 27	4			

TABLE 3.—MISCELLANEOUS DISEASES

Case	Diagnosis	Date, 1943	Protein tyrosine (arbitrary units)	Remarks
1	Chronic calculus cholecystitis	July 21	76	Icterus index 21; cephalin flocculation negative; bile ducts and liver normal at operation
2	Chronic calculus cholecystitis	July 22	75	Bile ducts and liver normal at operation
3	Chronic calculus cholecystitis	May 7	70	Bile ducts and liver normal at operation
4	Fever therapy for chronic gonorrhea	June 17	72	Before fever therapy
		June 23	76 90	After fever therapy
5	Fever therapy for chronic gonorrhea	June 17	83	Before fever therapy
			90	After fever therapy
		June 22	108	Patient jaundiced, icterus index 15
		July 1	83	Jaundice disappeared
6	Lobar pneumonia (Type I pneumococcus)	May 7	135	First reading obtained 4 days after crisis
		May 13	157	
		May 17	61	
7	Bronchopneumonia	May 8	91	"Atypical pneumonia"
		May 17	69	
8	Enteric fever	May 8	85	Mild case; blood culture, <i>B. typhosus</i>
		May 17	125	
		May 24	69	
9	Enteric fever	May 8	67	Mild case; blood culture, <i>B. typhosus</i>
		May 14	81	
		May 21	107	
		May 24	55	
10	Enteric fever	May 8	76	Mild case; blood culture, <i>B. typhosus</i>
		May 10	76	
11	Enteric fever	May 6	117	Mild case; blood culture, <i>B. paratyphosus A</i>
		May 17	84	
12	Meningococcic meningitis	May 8	81	
		May 10	99	
		May 17	87	
		May 27	81	
13	Meningococcic meningitis	May 22	70	
		May 27	56	
		May 31	82	
14	Meningococcic meningitis	July 5	102	
		July 12	94	
15	Influenza	May 17	98	Clinical diagnosis
16	Dengue fever	...	60	Clinical diagnosis
17	Acute pharyngitis	May 13	72	<i>Strep. pyogenes</i> and <i>Neisseria catarrhalis</i>
18	Acute tonsillitis	May 20	84	Alpha hemolytic streptococcus
19	Scrub typhus fever	June 16	73	Convalescent
		June 23	96	
20	Acute infectious mononucleosis	May 17	85	Heterophil agglutination 1:1280
21	Acute infectious mononucleosis	May 17	121	Heterophil agglutination 1:1280
		May 24	83	
22	Acute infectious mononucleosis	May 14	62	Heterophil agglutination 1:1280
		May 22	85	
23	Acute infectious mononucleosis	May 24	80	Heterophil agglutination 1:1280
		May 27	72	
24	Lymphogranuloma venereum	May 21	57	Virus isolated in eggs
		June 5	51	
25	Hookworm	May 7	83	Heavy infection
26	Hookworm	May 29	51	Moderate infection
27	Ascaris and whipworm	May 25	62	Moderate infection
28	Polycythemia vera	June 24	95	
		June 28	57	
		July 5	91	

malaria and were studied in a temperate, non-malarious region. Proske and Watson's subjects were exposed to reinfection while ours were not, since they were tested at least 3 months after their last possible exposure to infected mosquitoes. In the early part of the investigation, the blood protein tyrosine was high in many of our patients and it is possible that, if they had been studied immediately after leaving the malarious district, the elevation would have been higher and present in many more cases. The natural tendency of protein tyrosine to return to normal levels, which has been demonstrated in this paper, may well have been more obvious in our patients than in those that Proske and Watson studied.

It is apparent then that the seeming disparity of the 2 sets of observations may in reality indicate an important feature of malaria. If the blood protein tyrosine is increased in patients studied in endemic areas who are subject to reinfection, and not in patients who have been removed from such areas, then some alteration in blood proteins has occurred, the nature of which is as yet unknown. Comparable observations have been reported by Kopp and Solomon,⁵ who found

that in therapeutic malaria there is a transient impairment of liver function which disappears within 3 to 6 weeks after termination of malaria.

The observations in acute epidemic hepatitis suggest that determination of blood protein tyrosine may be a helpful laboratory procedure in the study of this disease. Presumably the test should be of help in studying other disorders of the liver and biliary tract, but unfortunately such a study was not possible because of lack of suitable clinical material in this hospital.

Summary. 1. Blood protein tyrosine (euglobulin) level was studied in patients with relatively acute malaria (*P. vivax*) 3 months after they had left the malarious district.

2. In these patients, and under the condition of the study the test was of limited diagnostic value, because 40% of those with positive blood films had a normal blood protein tyrosine. It was not of value in predicting relapses.

3. Elevated readings in patients with malaria returned to normal within a few months.

4. Blood protein tyrosine was elevated in many cases of acute epidemic hepatitis.

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THE URINARY EXCRETION OF METHIONINE IN LIVER DISORDER

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THE amino acid methionine, $\text{CH}_3\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$, has recently gained clinical recognition as a therapeutic agent in toxic hepatitis.^{3,8,9} The possibility of its usefulness in other sorts of liver disease has been claimed by some⁸ and denied by others.^{2,4,13} Therapeutic use of methionine is founded on experimental studies of numerous workers, among them Miller, Ross and Whipple,²⁰ Himsworth and Glynn^{14,15} and György.¹¹ The latter, based on his results with dietary experiments in rats, recommended the use of methionine as an adjuvant in the prevention and in the treatment of human liver disease. Du Vigneaud²³ demonstrated that methionine provides free methyl groups for the synthesis of choline and that it is of significance to sulfur and fat metabolism and to the problems of trans-methylation in the organism. The effectiveness of methionine in counteracting post-traumatic nitrogen loss in burned rats^{6,7} may constitute another property of this substance.

Beattie and his co-workers³ observed the beneficial effect of methionine in carbon tetrachloride poisoning and noticed the excretion of an excessive amount of urinary sulfur (equivalent to 65% of the sulfur given as methionine). They concluded that "the intimate cause of the liver disturbance induced by carbon tetrachloride is an abnormal metabolism of methionine and related compounds; and that in their patient it was specifically the administration of methionine which prevented further liver damage."

The present report deals with prelimi-

nary findings obtained in a study of the urinary excretion of methionine in various liver disorders.

First, the rate of the urinary excretion of methionine ingested by normal individuals orally was studied, and changes in urinary excretion due to the form of the absorbed methionine, either as crystals or as tablets containing a binder of unknown constitution, were investigated. Second, as renal impairment often accompanies liver disease, it was determined to what extent nephrosis and changes in urine volume would affect urinary methionine concentration.

Once these factors were known, methionine was given under various nutritional conditions and at various stages of liver disease to a patient with infectious hepatitis and to a patient with hepato-lenticular degeneration.

Materials and Methods. 1. *The Patients.* (a) Control investigations were done on young (30, 28, 32 years) healthy individuals who were doing their customary work in the laboratory during the experiments but adhered strictly to the necessary nutritional restrictions. In 1 instance a patient who was convalescent from severe burns was used as a control because his nutritional status at the time of the experiment was not significantly impaired and there was no evidence of liver disease.*

(b) The patient suffering from nephrosis was a 46 year old white female.† Albuminuria and edema had been present for 1½ years in this patient, whereas urine examinations prior to that period had been negative. The serum proteins in the past 1½ years varied from 4.5 to 3.5 gm. per 100 cc. and the

* Dr. F. H. L. Taylor's permission to use data obtained by his group on this patient is gratefully acknowledged.

† Patients (b) and (d) were private patients of Dr. H. Blotner whose cooperation in allowing study of these subjects is gratefully acknowledged.

albumin-globulin ratio was inverted. Cholesterol values were consistently around 600 mg. per 100 cc. of plasma. During the past year the patient had been kept on a high protein diet and was accustomed to weigh all her food as well as to measure her fluid intake and output. She was leading a semi-ambulatory life, doing some housework. During the experiment she was seen once a week and her drug, food and fluid intake was recorded with particular care.

(c) The patient with hepatolenticular degeneration has been described previously.^{16,17} He was a white boy of 19 having shown symptoms of hepatolenticular degeneration during several years. His liver function had been followed over a period of 14 months under various diets and it had been found that episodes of liver dysfunction could be provoked by restricting the dietary protein. The studies on methionine excretion in this patient were performed during such a period of induced hepatic disturbance.

(d) The patient suffering with infectious hepatitis was a white male of 38. He had always been healthy until March 1945, when, after the usual prodromata marked jaundice, right upper quadrant distress and enlargement of the liver appeared. Under high protein and vitamin diet, and bedrest the symptoms regressed within 6 weeks but 1 month later jaundice recurred with an icteric index of 100, 3+ bile in the urine and a 3+ cephalin flocculation. The stools were always of fairly normal color. The response to the dietary treatment was unsatisfactory and the general condition of the patient deteriorated, while the liver edge descended below the umbilicus. Beginning on Aug. 12, 1945, 5 gm. of methionine were given by mouth every day and the dose was later increased to 10 gm. For technical reasons the study of methionine excretion was begun only on September 7. Subjective improvement had been perceptible about 1 week earlier, while liver enlargement and laboratory findings remained unchanged. During the actual period of the experiment, the liver regressed to normal size and the icteric index fell. The cephalin flocculation became 2+. Methionine was discontinued after a total dose of 483.6 gm. had been

given. During the entire period of observation the diet had remained constant, the patient being hospitalized and a special dietitian being assigned to control his intake of food.

2. *Diets and Method of Methionine Administration.* It was kept in mind that various proteins of the diet contain different amounts of methionine. Methionine values of the usual constituents of hospital diets were not available and analysis of alimentary methionine was impossible for technical reasons. As a satisfactory compromise the sources of protein in all patients during the experiments were limited to casein, lean meat (lamb, beef and chicken) and the proportions of all of these constituents were kept fairly constant each day. The vegetable rations in each case were kept at comparable levels. Protein values were calculated from food tables.⁵ Regardless of changes of the amounts of proteins necessitated by the experiments, the diets were maintained at constant caloric levels by adding, in the case of low protein diets, corresponding amounts of carbohydrates and fat to make up for the omitted protein.

The d1-Methionine was given in the form of "Meonine"* tablets (containing 0.3 gm. of d1-methionine and a binder, the nature of which was unknown) in the case of the normal subjects, the patient with hepatolenticular degeneration and in the form of chemically pure crystals in the other patients. Unless otherwise specified the daily doses were fractionated into small amounts given every 3 hours from 8 A.M. to 8 P.M. in fruit juice or in water.

In the experiments on the variation of methionine excretion in normal subjects, the substance was given to 4 normal fasting individuals in the form of "Meonine" tablets with 200 cc. of water at 9 A.M. At 10 A.M. another 200 cc. of water were given. The urine was collected during the 12 hours preceding the experiment and its methionine content was measured. Urine was again collected at 12 noon, 3 hours after the ingestion of methionine, and the methionine contained in this 3 hour specimen was again determined. The same experiment was repeated on the same subjects several days later, using methionine crystals free of any binder.

* Part of the methionine used was supplied by Wyeth, Inc., through the cooperation of Dr. R. M. Johnson.

The excretion curves studied by Albanese² in normal persons were studied in 6 normal individuals, by measuring their urinary methionine before ingestion of 1.5 gm. of methionine ("Meonine" tablets) and $\frac{1}{2}$ hour, 1 hour, 2 hours and 3 hours thereafter. The amounts of fluid given during the experiment were identical to those used by Albanese.

in the form of tablets containing a binder or as powder by one and the same normal person are presented in Table 1. It appears that in some cases, methionine ingested in the form of pure crystals increases the amount of methionine excreted in the urine in a given time interval more markedly than does the same amount of

TABLE 1

Subject	Urinary methionine	
	12 hours before ingestion (mg./1 cc.)	3 hours after ingestion (mg./1 cc.)
<i>Effect of the Ingestion of 5 Gm. of Methionine in Tablets</i>		
F. H.	0 13	0.45
S. C.	0 24	0 39
B. O.	0 15	0 35
G. H.	0 16	0 18 (after 6 hours 0.36)
<i>Effect of the Ingestion of 5 Gm. of Methionine in Powder</i>		
F. H.	0 11	0 60
S. C.	0 18	0 41
B. O.	0 17	0.60
G. H.	0.14	0.18 (after 6 hours 0.40)

3. *Methionine Determination in Urine and Feces.* In all experiments except those described above all urine was collected in 24 hour specimens. Urine was preserved with 50 cc. of 15% hydrochloric acid plus 1 cc. of a 10% alcoholic thymol solution, and kept in the refrigerator. Methionine determinations were done within 7 days. This is a safe period for the preservation of methionine according to a personal communication from Albanese. The method of Albanese⁵ was used for urine methionine determinations. The same method was applied to fecal methionine after pulverization of the dried feces and resuspension of the material in distilled water.

4. *Other Laboratory Methods.* The total urinary nitrogen was determined by the macro-Kjeldahl method²¹ and 10% of the nitrogen intake were allowed for fecal nitrogen loss.

The cephalin flocculation test was performed according to Hanger's¹² technique and prothrombin time determinations were done by a modification of Quick's method.²² The icteric index was estimated by direct visual comparison of the serum to standards.

Results. 1. *Variations of Urinary Methionine Excretion Following Oral Administration of Tablets or Crystals.* Experiments on the excretion of methionine when taken

methionine, ingested by the same individual, in the form of tablets. It also appears that the amount excreted differs in different individuals. This point was further illustrated by repeating the excretion experiments of Albanese⁵ in 6 normal individuals. While 4 showed excretion curves comparable to those found by Albanese, 2 repeatedly had markedly slowed and prolonged excretion. (One of these was G. H. of Table 1.) -

2. *Methionine Excretion in a Normal Individual on Various Diets.* (Chart 1.) Albanese found that a normal adult "on a normal diet"²² excretes from 200 to 500 mg. of methionine in the urine in 24 hours. This does not significantly change when the subject is placed on a low protein diet (3.5 gm. of N₂) or when he is ingesting an isocaloric, high protein diet (30 gm. of N₂). When methionine is added, while the diet is poor in protein, a slight increase of urinary methionine excretion occurs. This is enhanced when the dietary protein is increased. The urinary methionine excretion immediately fell when methionine intake was diminished. The nitrogen balance under the

conditions of this experiment was not changed by the addition of methionine.

3. *Methionine Excretion in Nephrosis.* (Chart 2.) During a control period of 7 days on a diet of 3000 Calories containing about 150 gm. of protein this patient

excreted methionine in amounts comparable to those found in urine of a normal person. On 5 gm. of methionine tablets, the increase in urinary methionine was comparable to that observed in a normal person under similar conditions. On the

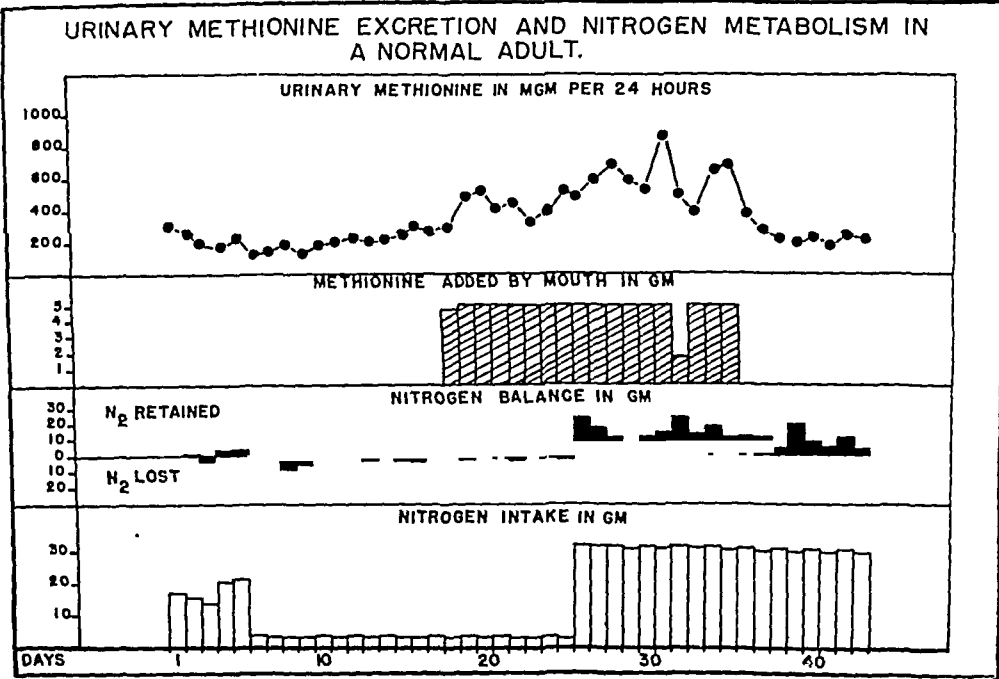


CHART 1.—Shows the changes in methionine excretion and nitrogen metabolism in a normal adult male of 30 on a diet of 3000 calories with varying amounts of protein.

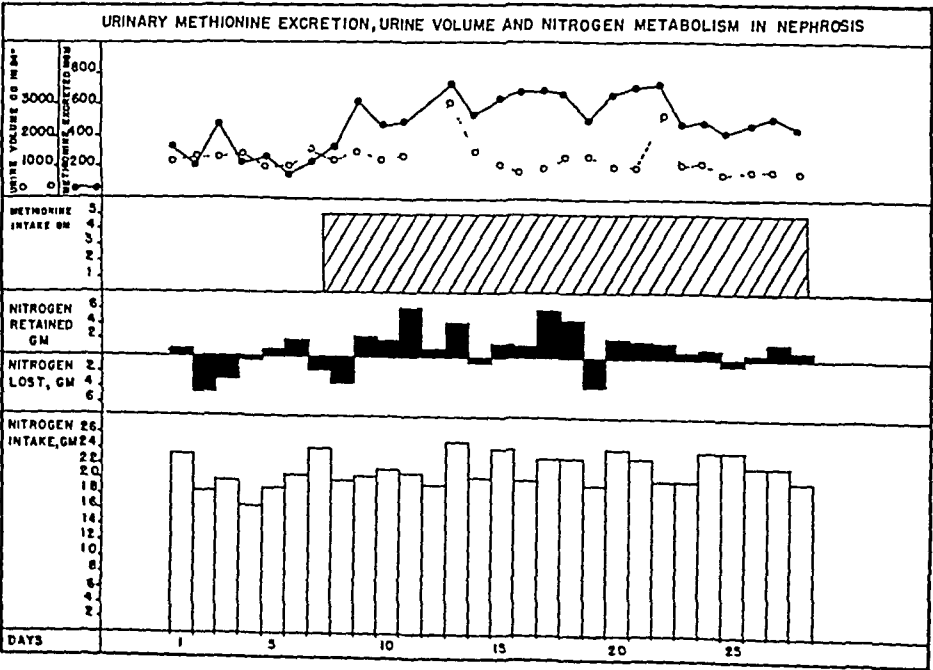


CHART 2.—Shows that there is no relation between urine volume and methionine excretion in a case of nephrosis maintained on a constant high protein diet.

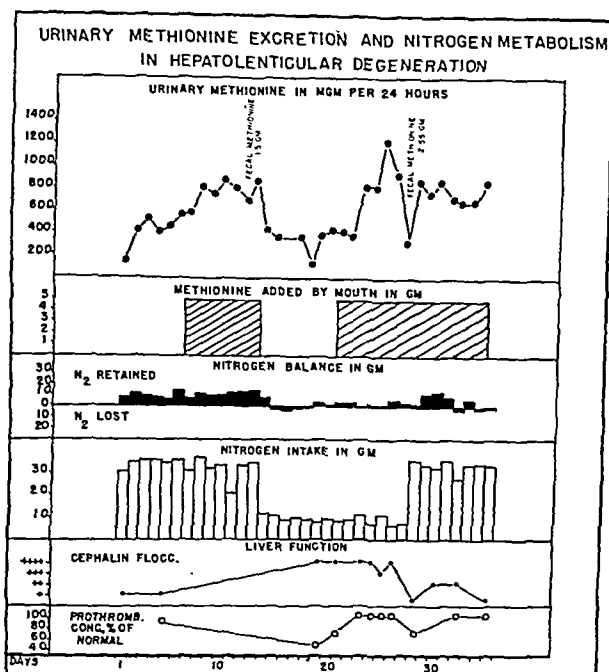


CHART 3.—Shows the changes in methionine excretion, nitrogen metabolism and liver function in a young adult male of 19 suffering from hepato-lenticular degeneration, maintained on a diet of 4000 calories with varying amounts of protein.

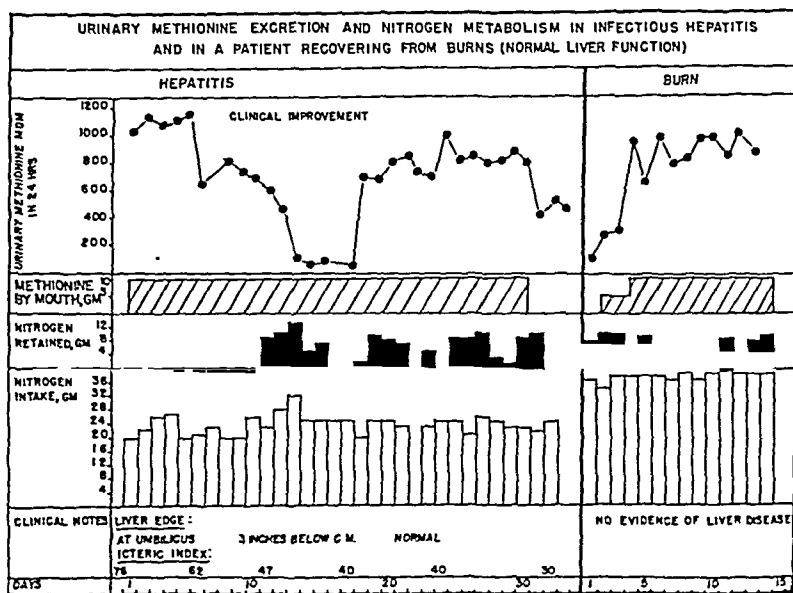


CHART 4.—Shows the changes of methionine excretion and nitrogen metabolism in a patient with infectious hepatitis during the period of recovery, on a constant high protein diet of 4000 calories. On the right side is shown the methionine excretion and nitrogen balance of a patient recovering from burns, on a constant high protein diet.

13th and on the 23rd day the urine volume was greatly increased following the injection of mercupurin. The changes of the methionine excretion did not parallel those of the urine volume. The nitrogen balance which varied somewhat during the experiment was more stable after the 20th day of the experiment. It is impossible to say whether methionine had anything to do with this change.

This experiment may be interpreted as meaning that the renal changes sometimes found in liver disease cannot be expected to affect significantly the urinary elimination of methionine.

4. *Methionine Excretion in Hepato-lenticular Degeneration.* (Chart 3.) In this patient acute liver failure followed the restriction of dietary protein which was reduced from 220 to 60 gm. while the total calories were maintained at 4000. In a preceding control period the administration of 5 gm. of methionine in tablet form daily had caused an increase of urinary methionine comparable or slightly higher than seen in the normal subject. During the low protein diet, when no methionine was given, 420 to 150 mg. of methionine were excreted, which is within the normal range. The addition of methionine to the low protein diet, at a time when liver function was poor, caused a more marked increase than that observed in the normal subject under similar conditions. This would have been even more accentuated had the patient not suffered from diarrhea which, at this point, nearly doubled his fecal methionine loss and greatly increased fecal nitrogen. The maximal methionine loss during this period was 3.75 gm. in 24 hours while it was only 2.4 gm. in 24 hours when methionine was given, together with a high protein diet and while liver function tests were normal.

5. *Methionine Excretion in Infectious Hepatitis.* (Chart 4.) In this patient methionine excretion studies were begun after he had received 5 gm. of methionine daily for 8 days and 10 gm. daily for 16 days. Clinical improvement had just begun when the study was started.

Throughout the experiment the patient was taking a high protein diet. During the first 5 days of the experiment his methionine excretion was between 1050 and 1165 mg. per day while in a control subject this value remained below 1000 mg. although the protein intake of the control was higher and to a great extent derived from casein, therefore rich in methionine. There was no evidence of liver damage in the control subject who was recovering from burns. The urinary methionine values in the patient with hepatitis fell below normal as he improved and approached levels found in the control subject as the patient's condition became stabilized. The nitrogen balance was not changed.

Discussion. The only previous studies on urinary methionine excretion are those of Albanese⁵ in normal subjects. However there is indirect evidence that in some conditions methionine loss in the urine is increased. Thus in the case of carbon tetrachloride poisoning Beattie *et al.*³ found an increased excretion of unoxidized sulfur (about 65 % of the amount of sulfur given as methionine). Miller¹⁹ reported that the feeding of methionine to protein depleted dogs caused an increase in urinary organic sulfur amounting to 15 to 40 % of the sulfur fed as methionine.

It is not known whether this methionine loss is due to the lack of certain components of protein, which might be necessary for the utilization of methionine as Gillman and Gillman¹⁰ have suggested, or whether the failure of the organism to fully utilize methionine is due to an impairment of certain metabolic functions of the liver. This latter hypothesis seems to be supported by the findings here presented. Whereas, the high methionine excretion in the patient with hepato-lenticular degeneration (Chart 3) was accompanied by liver dysfunction and by protein deficiency, the same phenomenon was observed with hepatitis while dietary proteins were adequate. In Miller's dogs¹⁹ the metabolic functions of the liver may well have been disturbed as serum phosphatase

and dye clearance may be impaired in dogs after relatively short times of protein starvation.¹⁸ In Beattie's patient the diet contained sufficient protein.

Furthermore, it has been shown that liver disease interferes with the metabolism of other amino acids. Bernhart and Schneider¹ showed this to be true for tyrosine and the studies of Abels, Kupel, Pack and Rhoads¹ demonstrated that the administration of glycine no longer increased urinary creatin and creatinine excretion when liver disease was present, even though free methyl groups were made available by simultaneous administration of choline.

The finding of increased urinary (and fecal) methionine excretion in liver dysfunction may be of practical importance as it may signify a defect in the proper utilization of methionine. This total methionine loss in the only instance where fecal methionine was determined amounted to 74% of the amount ingested and in Beattie's³ patient this amount was 65%. Larger therapeutic doses than those usually employed may therefore be needed, probably as much as 15 or 20 gm. a day. Some caution is indicated when elevating the dose, as it has been seen in this clinic that 30 gm. of methionine, taken in 1 single dose caused nausea and projectile vomiting. Some discouraging results reported with the therapeutic use of methionine for infectious hepatitis may have to be

interpreted with caution^{13,24} as much of the 5 gm. of methionine given may have been lost in urine and feces as methionine. It appears also important to start methionine therapy as early as possible, before liver impairment interferes with methionine utilization as seems to be suggested by our findings.

In studies dealing with methionine metabolism it should be kept in mind that the excretion in the urine of this amino acid is somewhat different, depending on the form in which it is given. The presence of a binder in tablets, such as in "Meonine," apparently delays absorption from the gastro-intestinal tract.

Summary and Conclusions. 1. Urinary methionine excretion in different subjects varies and is affected by the form (tablets or powder) in which methionine is given.

2. Urinary methionine excretion in 1 case was not influenced by renal changes of a nephrotic type nor by changes of the urine volume.

3. In 2 cases of liver dysfunction urinary methionine loss was found to be increased during the height of the disturbance and decreased as the patients improved. In 1 case this occurred independently of dietary factors.

4. Methionine seems to be lost in the urine in greater quantity during liver disease than normally and it seems desirable to use large doses as early as possible in the course of disorder of the liver.

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THE USE OF MOLYBDENIZED FERROUS SULFATE IN THE TREATMENT OF TRUE IRON DEFICIENCY ANEMIA OF PREGNANCY

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THE possible rôle of various trace elements, especially copper, in speeding recovery from certain experimental nutritional anemias, has been studied by a number of investigators. Up to the present time, however, copper alone has been convincingly demonstrated to be important in this regard, bringing about a better utilization of iron and quicker hemoglobin regeneration in animals with anemia induced by subsistence on a milk diet.⁵ Other elements such as manganese, cobalt and germanium have been reported to be ineffective.^{12,13}

Although the function of copper in hemopoiesis has been amply confirmed, it has been emphasized that this element allows a better utilization of iron only when there exists a combined deficiency of iron and copper such as results when the diet is made up exclusively of milk.³ Concerning its usefulness in clinical anemias, authoritative opinion is that medicinal copper is not necessary for treatment of anemias in man, with the exception of certain hypochromic nutritional anemias in young infants.^{4,7}

As far as one can learn from a careful search of the literature, molybdenum is not among the trace elements which have previously been studied from the standpoint of possible potentiation or catalysis of iron in the treatment of iron-deficiency anemias. In experimental studies of a molybdenized ferrous sulfate, which were undertaken and, on completion, will be

reported separately, early results suggested that treatment with this preparation accomplished an unusually rapid cure of milk-induced anemia in young rats. The present communication deals with preliminary clinical studies of the relative therapeutic efficacy of ferrous sulfate and of molybdenized ferrous sulfate* in hypochromic anemias in pregnancy.

Clinical Material.† Twenty-two co-operative, obstetric out-patients with true iron-deficiency anemia served as the clinical subjects of study. The diagnosis of true anemia was made in each of these patients on the basis of initial blood values that were below the minimum normal standards for pregnancy.

That the minimum normal values for hemoglobin and erythrocytes in the pregnant woman are substantially lower than those in the non-pregnant woman is now well established. This difference is attributed to the occurrence, during pregnancy, of a so-called physiologic anemia which does not respond to iron therapy, since it is not a true anemia but rather a hydremia. Bethell² believes that the lowest normal blood values for hemoglobin and erythrocytes in pregnancy are 11.3 gm. % and 3.70 million per c.mm., respectively. From their studies Adair and associates¹ conclude that the minimum standards for normal pregnancy are 10 gm. of hemoglobin, 3.36 million erythrocytes and 33 % of packed cell volume. Wolff and Limarzi¹⁵ state that the efficacy of iron therapy in

* Molybdenized ferrous sulfate is prepared by a special process, involving the co-precipitation of molybdenum sesquioxide and ferrous sulfate, with the production of a complex in which elemental molybdenum and ferrous iron are present in an approximate ratio of 1 to 15 parts, respectively. It was made available for clinical study in the form of tablets, each of which contained 3 mg. of molybdenum sesquioxide (approximately 2.5 mg. of elemental molybdenum) and 195 mg. of ferrous sulfate (approximately 40 mg. of ferrous iron). These tablets were analyzed for copper by spectrographic and colorimetric (dithiazone) methods. The results showed that this element was present to an extent not greater than 5 parts per 1 million.

† Clinical data were obtained from patients in the Obstetrical Clinic of the Orange Memorial Hospital, Orange, N. J., under supervision of the Obstetrical Department.

pregnant women with obviously true anemia—or with questionably true anemia in which hemoglobin and erythrocyte values are at or just below the minimum standards for pregnancy—may be predicted from the mean corpuscular hemoglobin concentration. According to the latter investigators, when this index is below the normal range of 32 to 36%, iron is indicated; when this index is normal, iron is contraindicated.

On initial examination, all of our 22 pregnant patients had hemoglobin values less than 10 gm. %; 21 had erythrocyte counts less than 3.36 million; one had an erythrocyte count of 3.8 million (with a hemoglobin value of 7.6 gm. %); all had subnormal mean corpuscular hemoglobin concentrations.

Method of Study. All laboratory studies were done by one special technician. Hemoglobin estimations were made with a Klett-Summerson photoelectric colorimeter; packed erythrocyte volume was determined by the modified Haden method; blood pipets, counting chambers and cover-glasses which were used had been certified by the U. S. Bureau of Standards.

Hematologic study before start of treatment consisted of hemoglobin and packed cell volume determinations, erythrocyte counts, total and differential leukocyte counts, and examinations of stained blood smears. Initial hematologic study of each patient was made during a pre-medication control period of at least 7 days. After treatment was begun, hemoglobin determinations and erythrocyte counts were made approximately at weekly intervals.

The 22 patients under study were divided into 2 numerically equal groups. Each patient in Group 1 received a daily dose of approximately 240 mg. of ferrous iron in the form of 6 tablets of ferrous sulfate; each patient in Group 2 received approximately the same daily dose of ferrous iron, plus 15 mg. of molybdenum, in the form of 6 tablets of molybdenized ferrous sulfate. Since it is now known that iron is clinically more efficacious in the bivalent rather than the trivalent form, precautions were taken to make certain that the iron content of tablets of both preparations remained in the bivalent state.

Supplies of medication, sufficient only for 2 weeks treatment, were regularly distributed to all patients. Prescribed dosage was adhered to in all cases, as far as could be determined from frequent inspection of supplies of distributed tablets and verification that estimated quantities of unused tablets corresponded with actual quantities.

Results. In Group 1 hemoglobin levels at start of treatment ranged from 7.6 to 9.8 (average 9.1) gm. %; in Group 2, these ranged from 7.6 to 9.4 (average 8.9) gm. %.

Following treatment for approximately the same length of time in both groups of patients, the hemoglobin levels in Group 1 were between 10.6 and 11.8 (average 11.2) gm. %; in Group 2 between 13.2 and 14.2 (average 13.5) gm. %. The average duration of treatment and weekly gain in hemoglobin were 6.9 weeks and 0.27 gm. % in Group 1; 6.2 weeks and 0.74 gm. % in Group 2. The foregoing data are listed in Table 1.

A more critical analysis of these results is facilitated by graphs of individual and group rates of hemoglobin formation. These are presented in Charts 1 to 3, examination of which reveals that the patients of Group 2, treated with molybdenized ferrous sulfate, experienced an unusually efficacious therapeutic response. The rate of hemoglobin formation in all patients of this group seemed to follow a distinctive pattern in which increments in hemoglobin concentration occurred with uniform rapidity until normal levels were almost attained. On the contrary, all patients in Group 1, receiving the same dosage of iron (but in the form of ferrous sulfate) as those in Group 2, exhibited an initially satisfactory therapeutic response followed by a progressively slower rate of hemoglobin regeneration, until hemoglobin values—still subnormal—were unaffected despite continued treatment with the same dose of ferrous sulfate. Curves of individual and average response to treatment in Group 1, therefore, showed an initial upward trend but gradually leveled off and became plateau-like while hemoglobin values were still subnormal.

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TABLE 1.—HEMOGLOBIN LEVELS AFTER TREATMENT WITH FERROUS SULFATE AND MOLYBDENIZED FERROUS SULFATE

Group 1.—Medication: Ferrous Sulfate				
Case No.	Initial Hb. (gm. %)	Initial M.C.H.C.*	Weeks of treatment	Subsequent Hb. (gm. %)
1	9 4	26	6	11.4
2	9 0	29	7	11 2
3	9 0	29	7	11.6
4	9 4	28	7	11 0
5	9 2	24	6	10 8
6	9 2	23	8	11.4
7	7 6	21	†	
8	9 4	25	8	11.0
9	9 8	29	7	11 8
10	8 8	29	7	10 8
11	9 8	24	6	10 6
Average	9.1	26	6 9	11 15

Average weekly gain in hemoglobin, 0.27 gm. %.

Group 2.—Medication: Molybdenized Ferrous Sulfate

12	9 4	28	7	13 6
13	9 2	30	8	13.1
14	9 4	27	6	13 7
15	9 0	23	6	13 6
16	9 0	23	6	13 2
17	9 0	25	5	13.4
18	9 4	23	5	13 4
19	9 0	25	6	14 2
20	8 2	28	7	13 4
21	9 0	30	6	13 2
22	7 6	26	6	14 2
Average	8 9	26	6 2	13 5

Average weekly gain in hemoglobin, 0.74 gm. %.

* M.C.H.C.—Mean Corpuscular Hemoglobin Concentration.

† Gastro-intestinal intolerance necessitated discontinuance of ferrous sulfate.

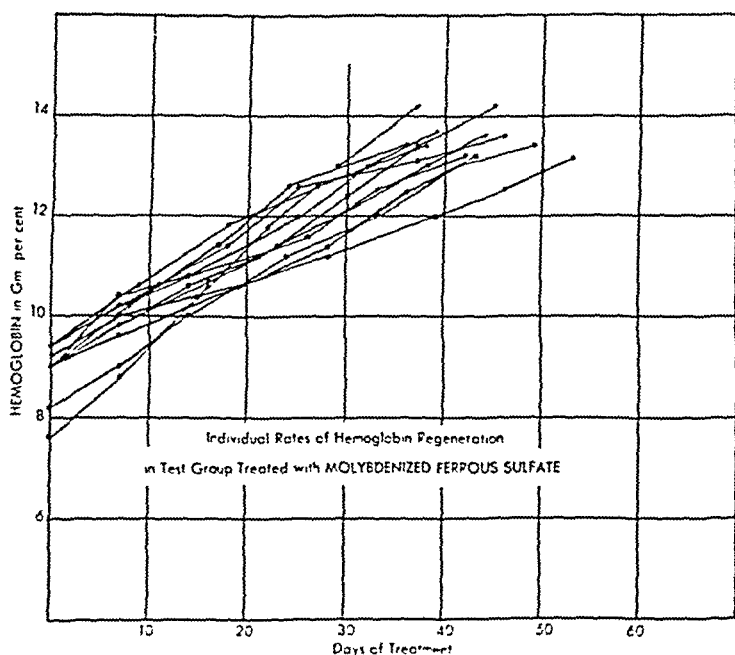


CHART 1

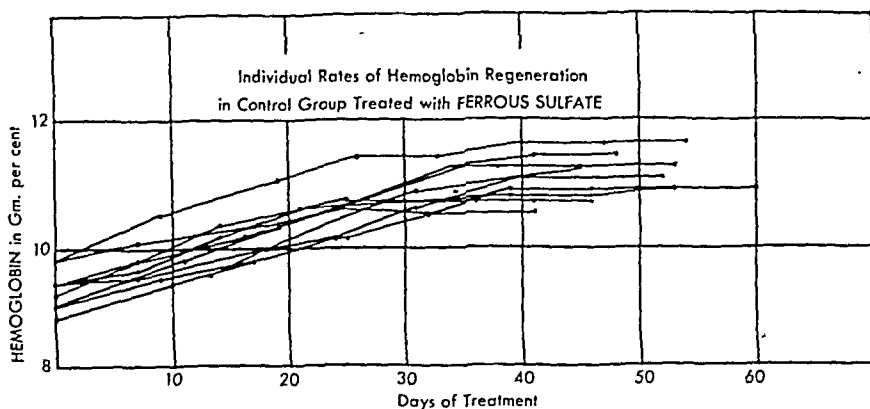


CHART 2

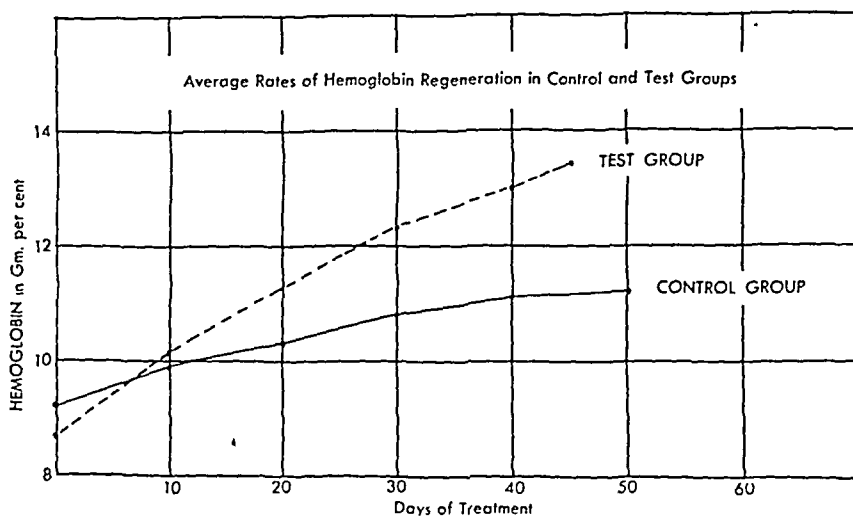


CHART 3

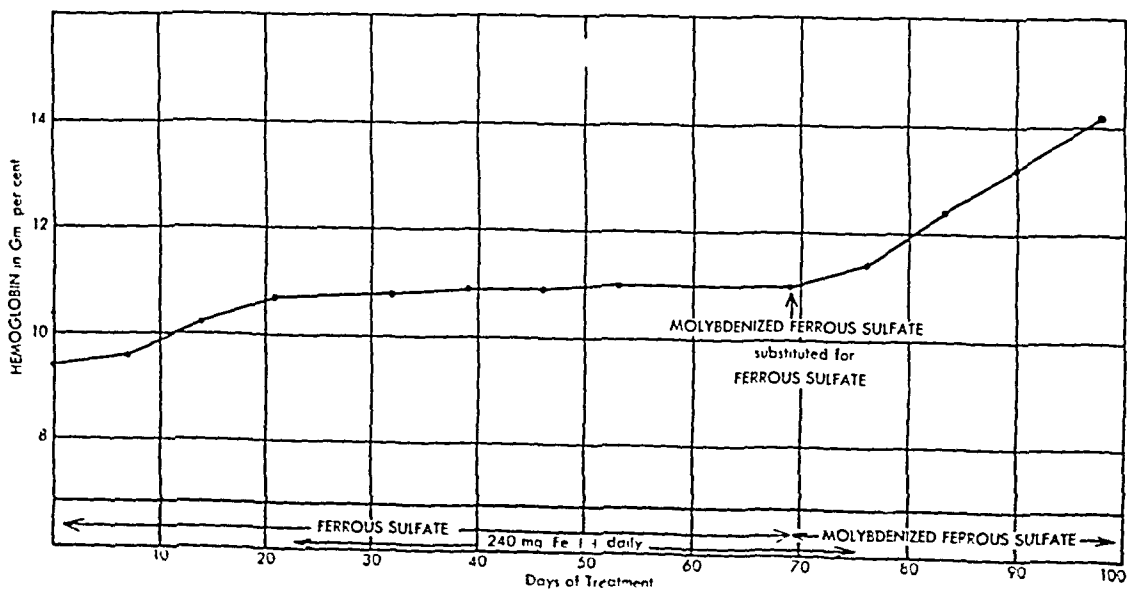


CHART 4

In all but 1 (Case 7) of the patients in Group 1, molybdenized ferrous sulfate was substituted for ferrous sulfate when the curve of therapeutic response had definitely assumed its plateau. Increases in hemoglobin values then occurred in each patient. Moreover, the rate of renewed hemoglobin formation was uniformly rapid until normal values were virtually reached. In Chart 4 is illustrated the renewed therapeutic response in Case 4, which occurred after molybdenized ferrous sulfate had been substituted for ferrous sulfate, and is generally typical of the pattern of renewed response exhibited by the remaining 9 patients similarly treated.

The response of erythrocytes to treatment in both Group 1 and Group 2 generally paralleled the rate of hemoglobin formation. Detailed discussion of the erythrocytic response seems unnecessary, since the rate of hemoglobin increase is the most reliable criterion of the efficacy of treatment in hypochromic anemias.

GASTRO-INTESTINAL TOLERANCE. Gastro-intestinal side effects were not noted in any of the patients during treatment with molybdenized ferrous sulfate. In Group 1, however, it was necessary to discontinue treatment with ferrous sulfate in 1 (Case 7) because of nausea which regularly followed ingestion of the medication. A second patient (Case 11) experienced mild gastro-intestinal irritation during early treatment with ferrous sulfate that disappeared as treatment was continued. Although no conclusions concerning comparative tolerance of the 2 iron preparations are justified because of the relatively small number of patients studied, it was interesting to observe that in Case 7 the patient experienced no side-effects from molybdenized ferrous sulfate with which she was successfully treated after her demonstrated intolerance of ferrous sulfate.

Comment. Arbitrary standards of satisfactory therapeutic response to iron medication, based on the rate of hemoglobin formation in uncomplicated hypochromic anemia, have been reported.⁶ Such standards, however, do not seem

applicable in hypochromic anemia of pregnancy. The latter condition is known to impair the response to iron,¹⁴ undoubtedly because one or more factors responsible for the anemia in pregnancy—such as the fetal requirements for blood-building materials—continue to be operative during the period of gestation. Consequently, a dosage of an iron preparation, sufficient to effect an optimal response in uncomplicated hypochromic anemia, might well be less effective in hypochromic anemia during pregnancy.

In the patients of Group 1, hemoglobin values were observed to rise, under treatment with ferrous sulfate, only to subnormal levels of 10.5 to 11.5 gm. %, despite continued treatment with ferrous sulfate in a daily dosage that one would expect to be fully effective in uncomplicated hypochromic anemia. That this subnormal plateau effect is the result of suboptimal dosage of ferrous sulfate was indicated by the renewed therapeutic response which was observed to follow a 50% increase in the daily intake of ferrous sulfate. Similarly, the average rate of hemoglobin formation in Group 1 (0.27 gm. % weekly for the entire therapeutic period), even if calculated for the first part of the therapeutic period during which hemoglobin values actually rose, would approximate only 0.4 gm. % weekly. This response is less than one would anticipate in uncomplicated anemia of comparable degree with the same quantity of ferrous sulfate.

In the patients of Group 2, however, the response to treatment with approximately the same dosage of ferrous iron, but in the form of molybdenized ferrous sulfate, was fully effective. It seems, therefore, that a daily dosage of approximately 240 mg. of bivalent iron was suboptimal, when administered in the form of ferrous sulfate, but was optimal, when given as molybdenized ferrous sulfate, in the 22 patients observed in this preliminary study.

Since recovery from hypochromic anemia was consistently rapid under treatment with molybdenized ferrous sulfate,

as compared with ferrous sulfate, the question of the mode of action of this newer preparation naturally arose. Speculating that the superior therapeutic effect of molybdenized ferrous sulfate might possibly result from an increased absorption of iron, we endeavored to study the comparative degree of gastro-intestinal absorption of this element, when administered as ferrous sulfate and as molybdenized ferrous sulfate, according to the method of Moore and associates.^{8,9} The latter investigators have reported that the degree (but not the total amount) of absorption of orally administered iron can be demonstrated through serial measurements of serum iron levels under stated experimental conditions. However, in 4 apparently normal adults we were unable to show by this method any significant difference in degree of iron absorption following oral administration of ferrous sulfate and of molybdenized ferrous sulfate.

In view of the foregoing, therefore, the thought is suggested that the apparently superior therapeutic activity of molybdenized ferrous sulfate in hypochromic anemias may be the result either of an *in vivo* catalysis of iron or, possibly, of a stimulation of erythropoiesis by molybdenum. Piper¹⁰ has reported that molybdenum is an essential element for plant growth. Ter Meulen's¹¹ finding that molybdenum occurs widely, although in minute amounts, in vegetable and animal materials also suggests the possible rôle of this trace element in animal and human nutrition.

Summary. 1. The hemopoietic activity of a specially processed molybdenized ferrous sulfate, as compared with that of ferrous sulfate, was studied in a series of 22 obstetric out-patients, all having

approximately the same degree of true iron-deficiency anemia and all receiving approximately the same daily dose of ferrous iron over the same periods of time.

2. In Group 1, treated with ferrous sulfate, the average gain in hemoglobin was 2.1 gm. % in 6.9 weeks; in Group 2, treated with molybdenized ferrous sulfate, the average gain in hemoglobin was 4.6 gm. % in 6.2 weeks.

3. The pattern of therapeutic response to molybdenized ferrous sulfate seemed to be distinctive, increases in hemoglobin occurring with uniform rapidity until normal values were almost attained; the therapeutic response to ferrous sulfate, although initially satisfactory, became progressively slower until hemoglobin values remained stationary at subnormal levels despite continuation of treatment.

4. The substitution of molybdenized ferrous sulfate for ferrous sulfate, when the latter compound no longer produced increments in subnormal hemoglobin values in Group 1, was followed by satisfactory renewal of hemoglobin formation until normal values were attained. Renewed therapeutic response was also effected by increasing the dosage of ferrous sulfate.

5. Patients treated with molybdenized ferrous sulfate tolerated the preparation extremely well; 1 patient, unable to tolerate ferrous sulfate, was treated effectively and without side-effects by molybdenized ferrous sulfate.

6. Molybdenized ferrous sulfate, therefore, seems to be an unusually efficacious, well-tolerated agent for treatment of true iron-deficiency anemia of pregnancy. Further study of its apparent advantages and its mode of action is in progress.

The assistance of Mrs. Blance M. Brant, who conducted all clinical laboratory tests, is gratefully acknowledged.

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THE EFFECT OF METHYLXANTHINES ON THE PROTHROMBIN TIME AND THE COAGULATION OF THE BLOOD

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IN the course of investigations concerning diuresis, Nonnenbruch and Szyszka discovered that in rabbits the coagulability of the blood increased so much after an intravenous injection of a mixture of theophylline and ethylenediamine that it was difficult to obtain blood from the ear vein because of the immediate clotting. This led to studies by these authors¹⁰ and others^{1,7} of the effect of theophylline with ethylenediamine on the coagulation of blood in animals and man. The original observations were confirmed and this drug was recommended as a coagulant in cases with hemorrhagic diathesis.^{8,10,11} The effect was attributed primarily to the action of the ethylenediamine.¹⁰ Recently, it was shown that the methylxanthines (theophylline, theobromine and caffeine) exert a distinct effect on the prothrombin time and shorten it to a degree which may have clinical importance.⁴

We investigated the effect on prothrombin coagulation time and on plasma coagulation in man produced by the intravenous injection of theophylline and ethylenediamine (aminophylline) and theophylline with sodium acetate and by the oral administration of several methylxanthines.

Method of Study. All subjects were hospitalized on the medical wards. Care was taken to eliminate all patients who had any hepatic disease and those who were receiving vitamin K, salicylates, quinidine or any product which influences the prothrombin concentration or the clotting mechanism. With 2 exceptions all were males because in them venipuncture is much easier and also because they are less apt to object to the repeated venipuncture.

Control (normal) prothrombin and plasma coagulation times were first done. Then, the patients were given 0.5 gm. of aminophylline or theophylline with sodium acetate intravenously; blood was taken 1, 2, 3 and 24 hours after the injection for estimation of the prothrombin and plasma coagulation times. Those who received the methylxanthines by mouth were tested after periods of 24, 48, 72, 96 and 120 hours.

The prothrombin test was done as follows: one-tenth of 1 cc. of a 10% solution of potassium oxalate was placed into a graduated centrifuge tube. A venipuncture was done with as little trauma as possible and blood drawn into an absolutely dry syringe. Enough blood was added to the centrifuge tube to make up 4.5 cc. of the mixture. This was centrifuged at 1500 r.p.m. for 5 minutes and the oxalated plasma pipetted into a small dry test-tube (75 by 10 mm.). The test was done immediately because it is known that prothrombin is very labile and disintegrates on standing.

The Fullerton modification of the Quick method was employed.⁵ Two-tenths of 1 cc. of oxalated plasma was pipetted into a test-tube to which was added 0.2 cc. of Russell viper venom.* The tube was placed into a water bath at 37° C. and 0.2 cc. of 1.11% solution of calcium chloride added. The moment the calcium chloride made contact with the solution, a stop-watch was started. The tube was agitated gently and the first appearance of a fibrin-web was taken as the end-point. All tests were performed in duplicate and a mean computed. This method yielded an average control prothrombin time of 20.3 seconds.

The plasma coagulation tests were done in parallel with the prothrombin. A modification of the method first used by Cheney, who in turn had modified the original Howell

* We are indebted to Burroughs Wellcome & Co., for our supply of Stypven, Russell viper venom.

prothrombin concentration test, was employed.³ These tests were done at laboratory room temperature which varied between 25° and 28° C. Two-tenths of 1 cc. of oxalated plasma (prepared as in the prothrombin time test) was pipetted into a test-tube (75 by 10 mm.) and 0.2 cc. of 1.11% of calcium chloride added. The tube was agitated gently for 10 seconds, to assure thorough mixing, and then tilted to the horizontal every 30 seconds and oftener as coagulation began. When the solution did not flow on tilting the test-tube to the horizontal, this was considered the end-point.

prothrombin time was shortened by more than 3 seconds. In 21 cases, the prothrombin time was determined 1 hour following the injection and in 18 of these a distinct shortening appeared within this time. The most marked shortening was obtained 1 hour after the injection in 8 cases, 2 hours after the injection in 3 cases and in 3 hours after the injection in 9 cases.

Figure 1 shows a graph of those cases that had the greatest decrease in the pro-

TABLE 1.—THE EFFECT OF INTRAVENOUS AMINOPHYLLINE (0.5 GM.) ON THE PROTHROMBIN TIME

Prothrombin time (sec.)									
Name	Age	Sex	Control	Hours after taking of drug				Maximum change	Diagnosis
				1	2	3	24		
W. U.	54	M	26.0	16.8	17.4	21.6	19.0	9.2	Peptic ulcer
B. B.	32	M	20.0	20.2	17.0	15.0	17.0	5.0	Peptic ulcer
J. D.	65	M	21.2	20.0	19.0	13.2	18.6	8.0	Brain tumor
D. G.	45	M	21.0	19.0	20.0	15.0	20.0	6.0	Diabetes
A. B.	79	M	22.6	21.4	24.8	15.8	17.8	6.8	Pneumonia
A. B.	79	M	16.0	18.0	14.0	16.4	16.6	2.0	Hypertension
W. G.	36	M	17.2	14.8	15.4	17.0	12.6	4.6	Lues
L. H.	28	M	20.0	13.0	18.7	16.0	18.0	7.0	Pleurisy
B. H.	79	M	17.0	15.8	18.2	17.6	16.8	1.2	Bronchitis
J. A.	50	M	20.8	19.0	19.0	18.8	18.0	2.8	Coronary thrombosis
A. T.	38	M	21.0	23.0	29.0	28.8	35.0	-14.0	Colitis
V. K.	16	M	18.2	20.6	18.0	16.4	20.6	1.8	Pneumonia
J. L.	47	M	22.2	20.0	19.4	19.8	20.2	2.8	Lung tumor
A. R.	37	M	18.6	17.2	15.8	16.6	19.8	2.8	Amebiasis
J. B.	66	M	17.4	15.6	18.2	12.4	20.8	5.0	Bronchiogenic carcinoma
J. U.	25	M	18.8	18.4	17.0	13.0	18.6	5.8	Schistosomiasis
C. J.	73	M	21.2	17.4	16.4	14.0	20.0	6.2	Prostatic hypertrophy
H. G.	49	M	17.2	15.0	15.0	15.2	17.2	2.2	Empyema
A. G.	52	M	19.0	18.0	14.5	14.2	17.0	5.8	Coronary thrombosis
H. T.	14	M	19.0	16.4	16.4	17.5	20.2	4.8	Pneumonia
R. W.	77	F	24.0	18.5	18.5	..	16.8	7.2	Ptoxis, right kidney
G. M.	31	F	23.5	..	14.0	..	10.0	9.5	Petit mal

This test is somewhat empiric, as no attempt was made to determine the optimal amount of calcium chloride necessary to give the shortest coagulation time; however, our normal controls had an average of 4.9 minutes and this compares favorably with Cheney's average of 5.2 minutes.

Results. *Intravenous Injection of Aminophylline.* The effect of intravenous aminophylline was investigated in 22 patients. The data on prothrombin time are compiled in Table 1. The ages of the patients varied between 14 and 79 years. Of 22 cases, 21 showed a shortening of the prothrombin times following the injection. The 1 exception concerned a 38 year old male with colitis; he showed a steady increase of 14 seconds in 24 hours. The smallest change was 1.2 seconds and the greatest was 9.5 seconds. In 14 cases, the

thrombin time at the end of 3 hours. The results are uniform. In 2 instances, Cases R. W. and G. M., the prothrombin time was determined 6 hours after injection and in both there was a distinct tendency to return to control levels as compared to the value obtained after 3 hours.

The results of the measurements of the plasma coagulation times are compiled in Table 2. The first 20 cases in Table 1 were also used for this investigation. The coagulation time was shortened following the injection of aminophylline in 18 of the 20 cases studied. In 2 cases it remained unchanged after 1 hour and then gradually increased. In 15 cases the shortening of the coagulation time was greater than 1 minute. Even in the case of colitis (A. T.), in which the prothrom-

TIME AND COAGULATION OF BLOOD

at the end of 3 hours. In 3 cases the shortest coagulation time was found 24 hours after the injection. In 24 hours, the coagulation time was distinctly increased in 16 cases and in 12 of these

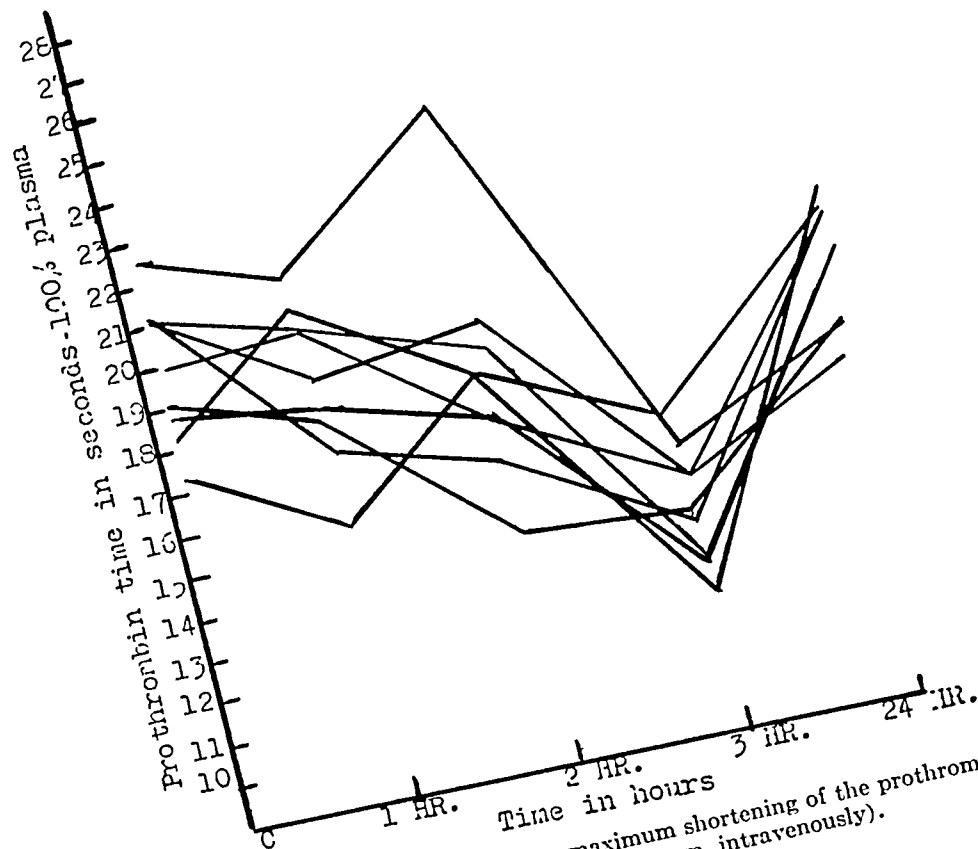


FIG. 1.—Showing all those cases that had a maximum shortening of the prothrombin time in 3 hours (aminophylline, 0.5 gm. intravenously).

Plasma coagulation time (min.)	Hours after taking of dose	
	2	3
26		

Name	Age	Sex	Plasma coagulation time (min.)					Maximum change	Diagnosis
			Control	Hours after taking of drug					
				1	2	3	24		
W. U.	54	M	2.4	2.5	1.6	2.6	2.0	0.8	Peptic ulcer
B. B.	32	M	2.0	2.0	2.0	2.2	3.8	-1.3	Peptic ulcer
J. D.	65	M	4.0	3.4	3.3	3.6	4.0	0.7	Brain tumor
D. G.	45	M	3.9	4.1	6.9	3.1	3.3	0.8	Diabetes
A. B.	79	M	7.3	3.1	6.0	4.1	3.7	3.6	Pneumonia
A. B.	79	M	3.0	3.3	2.1	2.0	3.0	1.1	Hypertension
W. G.	36	M	4.9	4.0	2.0	2.5	4.9	3.0	Lues
L. H.	28	M	6.1	2.7	3.6	1.9	2.2	1.0	Pleurisy
B. H.	79	M	5.2	3.3	2.2	4.2	2.5	3.0	Bronchitis
J. A.	50	M	3.3	3.0	3.8	3.0	5.0	1.8	Coronary thrombosis
A. T.	38	M	3.0	3.0	3.4	2.7	3.4	3.6	Colitis
V. K.	16	M	3.8	3.0	4.0	3.8	5.0	2.2	Pneumonia
J. L.	47	M	5.0	3.5	2.0	3.6	3.5	1.2	Lung tumor
A. R.	37	M	5.3	4.7	4.0	3.3	5.0	1.3	Amebiasis
J. B.	66	M	5.2	3.0	2.6	3.2	3.5	-1.0	Bronchiogenic carcinoma
J. U.	23	M	7.0	5.3	3.7	3.7	4.0	1.5	Schistosomiasis
C. J.	73	M	4.7	2.3	2.0	2.3	5.0	2.7	Hypertrophy prostate
H. G.	49	M					5.3	2.8	Empyema
A. G.	52	M					7.0	3.2	Coronary thrombosis
H. T.	14	M					1.5	4.5	Pneumonia
							6.3	2.7	

the control values were reached or surpassed.

Intravenous Injection of Theophylline With Sodium Acetate. The results on the 12 cases studied are compiled in Tables 3 and 4. Although the ages ranged from 22 to 85 years, 11 of the patients were 50 or more years old. Eleven of the 12 subjects showed a significant shortening of the prothrombin time; the smallest was 0.4 second, as compared with 1.2 seconds for aminophylline, and the greatest shortening was 6.8 seconds as compared with

infarct died from multiple embolic phenomena, before the experiment could be completed. It is interesting to note that although he had a very short control prothrombin time (14 seconds), the hyperprothrombinemia increased during the embolic episode with a shortening of the prothrombin time to 8.4 seconds.

Eight of the 12 subjects showed a shortening of the plasma coagulation. This ranged from 0.8 to 5 minutes. Four of the patients exhibited a lengthening of the coagulation time for which no explana-

TABLE 3.—THE EFFECT OF (I. V.) THEOPHYLLINE SODIUM ACETATE (0.5 GM.) ON PROTHROMBIN TIME

Name	Age	Sex	Control	Hours after taking of drug				Maximum change	Diagnosis
				1	2	3	24		
A. B.	56	M	22 0	23 2	21 2	21 4	17 4	4 6	Arthritis
A. L.	54	M	19 2	21 6	20 0	20 6	18 8	0 4	Cerebral hemorrhage
C. J.	73	M	22 8	19.8	18 4	21 0	16 8	6 0	Prostatic hypertrophy
M. J.	67	M	14 0	13 2	8 4	16 8		5 6	Pulmonary infarct
J. A.	50	M	20 4	18 2	16 0	18 6	14 6	5 8	Coronary thrombosis
E. J.	22	M	18 6	17 0	22 5	21 2	14 8	3 8	Arthritis
J. L.	55	M	20 0	19 6	17 2	19 8	21 2	2 8	Cerebral hemorrhage
W. M.	70	M	22 4	20 8	16 0	18 4	17 4	6 4	Arthritis
J. D.	85	M	20 6	17 8	15 6	17 2	21 6	5 0	Coronary sclerosis
J. R.	35	M	22 0	15 2	16 2	18 0	22 0	6 8	Bleeding peptic ulcer
S. P.	68	M	22 0	19 6	22 0	20 4	21 2	2 4	Hemiplegia
A. S.	63	M	20 8	22 0	21 6	19 0	22 6	1 8	Parkinson's disease

TABLE 4.—THE EFFECT OF (I. V.) THEOPHYLLINE SODIUM ACETATE (0.5 GM.) ON COAGULATION TIME

Name	Age	Sex	Control	Hours after taking of drug				Maximum change	Diagnosis
				1	2	3	24		
A. B.	56	M	8 0	8 3	9 0	8 0		11 -1	Arthritis
A. L.	54	M	6 3	7 3	5 5	6 0		0 5	Cerebral hemorrhage
C. J.	73	M	6 0	7 0	11 5	7 0		-5 5	Prostatic hypertrophy
M. J.	67	M	2 5	2 8	3 0	3 7		-1 2	Pulmonary infarct
J. A.	50	M	5 0	5 8	4 0	6 0	2 3	-2 7	Coronary thrombosis
E. J.	22	M	4 5	4 0	6 5	5 0	1 5	3 0	Arthritis
J. L.	55	M	8 5	7 0	8 5	3 5	6 5	5 0	Cerebral hemorrhage
W. M.	70	M	3 5	9 0	9 0	3 5	4 0	-5 5	Arthritis
J. D.	85	M	8 0	7 0	7 5	3 5	10 5	4 5	Coronary sclerosis
J. R.	35	M	6 5	4 5	3 0	3 3	5 5	3 5	Bleeding peptic ulcer
S. P.	68	M	5 5	3 3	5 0	4 7	9 0	2 2	Hemiplegia
A. S.	63	M	6 0	3 5	6 5	7 5	7 5	2 5	Parkinson's disease

9.5 seconds for aminophylline. The average decrease in prothrombin time was 4.29 seconds as compared to 4.7 seconds for aminophylline. In 5 of the 12 patients, the greatest hyperprothrombinemic effect of the drug occurred after 24 hours; in 4 in 2 hours; in 2 in the 1st hour; and in 1 at the 3rd hour after injection. This is in contrast to aminophylline where the majority of the patients (14) had returned to their control levels after 24 hours. Seven of the patients had a significant shortening as early as 1 hour. One of the patients (M. J.) who had a pulmonary

infarct died from multiple embolic phenomena, before the experiment could be completed. Of those who had a decrease in time, the average was 3.03 minutes as compared to 2.1 minutes for aminophylline. The maximum decrease was evenly divided among the 1, 2, 3 and 24 hour periods after injection.

No correlation between the effect on the prothrombin and coagulation times is possible; those cases (C. J., M. J., W. M., A. S.) with a lengthening of the coagulation time showed the most significant shortening of the prothrombin time.

Patients C. J. and J. A. were tested with both aminophylline and theophylline with

sodium acetate. The former showed a decreased prothrombin time of 6.2 seconds with aminophylline and 6 seconds with theophylline with sodium acetate and the latter a shortening of 2.8 seconds with aminophylline and 5.8 seconds with theophylline with sodium acetate. C. J. showed a lessened coagulation time of 2.8 minutes with aminophylline and an increased time of 1 to 5.5 minutes with theophylline with sodium acetate, while

showed a significant shortening in the prothrombin time which averaged 4.65 seconds as compared to intravenous aminophylline with 4.7 seconds, and intravenous theophylline with sodium acetate with 4.29 seconds. Six of the subjects were tested 24 hours after ingestion of the drug, and 3 showed a detectable hyperprothrombinemia. In 1 case (N. P.), the maximum shortening occurred in the 24 hour period. Patients H. G. and A. G.

TABLE 5.—THE EFFECT OF THE METHYLXANTHINES (BY MOUTH) ON PROTHROMBIN TIME

Name	Age	Methylxanthines	Dose (gm. t.i.d.)	Prothrombin time (sec.)					Maximum change	Diagnosis
				Control	24	48	72	96		
H. G.	49	Theobromine	1.0	20.0	15.3	..	4.7	Empyema
A. G.	52	Theobromine	1.0	21.0	14.5	..	6.5	Coron. thrombosis
T. H.	73	Aminophyllin	0.5	22.3	20.5	16.5	5.8	Arteriosclerosis
J. C.	80	Aminophyllin	0.5	24.5	24.8	18.4	6.1	Arteriosclerosis
J. C.	53	Theobromine	1.0	21.6	22.4	22.0	-0.8	Neurosyphilis
A. S.	47	Theobromine	0.5	20.6	19.6	19.8	15.7	20.4	6.0	Beri-beri, heart
L. B.	41	Theobromine	1.0	18.2	19.2	19.8	18.4	16.4	2.5	Virus pneumonia
J. Y.	59	Theobromine	1.0	21.4	14.9	18.4	17.5	19.0	5.0	Coronary sclerosis
N. P.	57	Theobromine	1.0	20.2	5.3	Abdominal mass
I. K.	48	Sodium acetate	0.5	18.0	2.6	Hypertension
S. S.	31	Theobromine	1.0	18.2	-0.8	Coron. thrombosis
T. Q.	57	Sodium acetate	1.0	19.0	20.0	19.8	23.0	..	-4.0	Mitral stenosis
U. M.	62	Theobromine	1.0	16.2	18.4	16.8	18.4	..	-2.2	Gastric carcinoma
M. D.	73	Theobromine	1.0	19.4	18.0	17.4	20.2	..	2.0	Hemiplegia

TABLE 6.—THE EFFECT OF THE METHYLXANTHINES (BY MOUTH) ON PLASMA COAGULATION TIME

Name	Age	Methylxanthines	Dose (gm. t.i.d.)	Coagulation time (min.)					Maximum change	Diagnosis
				Control	24	48	72	96		
H. G.	49	Theobromine	1.0	7.0	6.0	..	1.0	Empyema
A. G.	52	Theobromine	1.0	6.3	5.5	..	0.8	Coron. thrombosis
T. H.	73	Aminophyllin	0.5	5.0	7.0	7.0	-2.0	Arteriosclerosis
J. C.	80	Aminophyllin	0.5	4.0	8.5	7.4	-4.5	Arteriosclerosis
J. C.	53	Theobromine	1.0	7.0	9.0	8.0	-5.5	Neurosyphilis
A. S.	47	Theobromine	1.0	5.8	7.0	7.5	6.0	5.7	3.0	Beri-beri, heart
L. B.	41	Theobromine	1.0	5.5	5.3	6.5	5.5	6.5	-1.7	Virus pneumonia
J. Y.	59	Theobromine	1.0	4.0	2.5	5.0	7.5	6.0	0.2	Coron. sclerosis
N. P.	57	Theobromine	1.0	4.0	1.5	Abdominal mass
L. K.	48	Sodio-acetate	0.5	3.0	0.5	Hypertension
S. S.	31	Theobromine	1.0	7.5	6.0	3.0	7.0	..	-2.7	Coron. thrombosis
T. Q.	57	Sodio-acetate	1.0	3.5	4.0	6.3	7.0	..	4.5	Mitral stenosis
U. M.	62	Theobromine	1.0	7.5	2.0	3.0	5.5	..	-3.5	Gastric carcinoma
M. D.	73	Theobromine	1.0	5.5	Hemiplegia

J. A. had a decreased time of 2.5 minutes with the former drug and a shortened time of 2.7 minutes with the latter medication.

Effect of Methylxanthines by Mouth. This study was done on 14 patients who received the methylxanthines in the form of capsules of theobromine, 1 gm. 3 times daily, tablets of aminophylline, 0.5 gm., 3 times daily, and enteric-coated tablets of theobromine sodium acetate, 0.5 gm., 3 times daily. The results are summarized in Tables 5 and 6. Ten of the 14 patients

received theobromine 1 gm., 3 times daily, and were tested 72 hours later. They had also been previously tested with intravenous aminophylline. The patient, H. G., had a lessening of the prothrombin time of 2.2 seconds following the intravenous administration of aminophylline as compared with 4.7 seconds after the oral medication. The patient, A. G., showed a decrease of 5.8 seconds with intravenous aminophylline as compared with 6.5 seconds for theobromine by

mouth. Three patients were studied on 4 consecutive days; 1 showed a maximum reduction on the 3rd day, 1 on the 4th and 1 on the 1st day. In 4 patients the prolongation of the prothrombin time varied from 0.8 to 4 seconds. This lengthening occurred with all 3 of the drugs used.

A study of Table 5 indicates that all 3 drugs were equally effective in reducing the prothrombin time, and that this reduction equals that produced by the intravenous methylxanthines.

Seven of the 14 patients developed a shortened coagulation time ranging from 0.5 to 5.5 minutes. The other 7 had a lengthened coagulation time of 1 to 5.5 minutes. Again, no parallelism between the prothrombin and clotting times can be drawn for those who had a decrease in the prothrombin time showed an increased coagulation time; the reverse was also true. In 3 instances (U. M., S. S., and A. S.) prolongation of both the prothrombin and clotting times occurred after the oral administration of methylxanthines.

Discussion. The investigations reported above show a definite shortening of the prothrombin time and of the plasma coagulation time following an intravenous injection of aminophylline. The changes were often found within 1 hour after the injection and they seem to reach a maximum 4 to 5 hours later. Often the values were still abnormal after 24 hours. A cumulative effect of injections administered daily for some time is therefore possible.

The intravenous injection of theophylline with sodium acetate had a similar effect. Therefore the action is not bound to the ethylenediamine¹⁰ which is used as a solvent for the theophylline.

The increased coagulability of the blood following the administration of aminophylline led to the recommendation of this drug as a styptic agent.

Aminophylline and other methylxanthines are, however, widely employed for their diuretic effect in bedridden cardiac patients. The danger of thrombosis in

the veins of the lower extremities of such patients is great, since anatomic evidence shows that it occurs in more than 50% of all autopsies in a general hospital.^{6,9} Aminophylline is also used frequently in patients with coronary sclerosis who may at any time develop a coronary thrombosis. It is often given for Cheyne-Stokes respiration, where it has a specific effect. It is administered in cardiac asthma and is given to dilate the coronary arteries.² It is not probable that the changes in the prothrombin time *per se* will have a detrimental effect in a patient with a normal circulation who is not confined to bed. The possibility, however, that the increased coagulability of the blood will augment the danger of venous thrombosis in the bedridden patient or the risk of coronary thrombosis in a patient with coronary sclerosis merits serious consideration.

During the last 20 years we have given aminophylline and theophylline with sodium acetate intravenously to a large number of patients. Occasionally coronary thrombosis appeared during the treatment but this was considered to be an accidental event. Perhaps now that attention has been directed to a possible causal relationship between such an accident and the administration of methylxanthines more observations will be available of such accidents.

Perhaps the administration of dicoumarin will be necessary in those patients in whom the methylxanthines are indicated.⁴

The cause of the hyperprothrombinemia following the administration of methylxanthines is unknown. A functional stimulation of the hepatic tissue has been suggested as a causative factor.⁴

Summary and Conclusions. Shortening of the prothrombin time and of the plasma coagulation time was observed in man following the intravenous administration of aminophylline and theophylline with sodium acetate. Methylxanthines, given orally have a similar effect.

The clinical implications of these findings are discussed.

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BLOOD LEVELS OF PENICILLIN AFTER VARIOUS FORMS OF ORAL ADMINISTRATION

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THE destructive action of the acid of gastric juice¹ is the chief factor opposing effective administration of penicillin by mouth. Many methods are applicable in part in overcoming this factor.

We have investigated the following methods during the past year:

A. The administration of penicillin on an empty stomach when gastric emptying will be rapid and acidity low.

B. The use of various alkalis to bring the pH of the stomach contents up toward the 5 to 7 range where penicillin is most stable. We have confirmed the work of György *et al.*² and Charney *et al.*³ in this respect.

ing would be one completely insoluble in acid and readily soluble in alkali. Of various coatings tried, we have found a resin⁴ most satisfactory.

E. The use of an adsorbing agent that would release penicillin slowly.

F. Combinations of the above methods.

Procedure. Blood penicillin levels have been determined by the Fleming slide cell method⁵ and urine concentrations by the Oxford cup method.¹

One group of subjects were given the drug in aqueous solution without any accompanying medication. Part of these subjects (Table 1) received the drug on an empty stomach and part received the drug after a meal. It is obvious that the presence of

TABLE 1.—BLOOD LEVELS OF PENICILLIN AFTER THE ORAL ADMINISTRATION OF 100,000 UNITS OF CALCIUM PENICILLIN IN TAP WATER

Subject	Blood levels (Oxford units per cc. of blood)						
	½ hr.	1 hr.	1½ hrs.	2 hrs.	3 hrs.	4 hrs.	% excretion
S*	..	0 06	0 03	0	0	.	6 6
S*	..	0 12	..	0 03	0	.	15 9
W*	0 12	0 12	0	0 06	0	.	1 9
S†	0	0	0	0	0	.	1 5
W†	..	0	0	0	0	.	1 2c
S†	..	0 12	0	0	0	.	0 48
W†	0 03	0	0	0	0	.	21 2
R‡	..	0 24	..	.	0 24	0 03	

* Empty stomach.

† Full stomach.

‡ Pernicious anemia.

C. The use of a substance that will hold penicillin in a suspension or solution that does not mix readily with the gastric contents and that will thus protect it from the acid and serve to slow the absorption. Libby *et al.*⁷ found that cotton-seed oil served this purpose to some extent. We have found a mixture of lanolin and sesame oil⁸ and also cocoa butter to be suitable.

D. The use of an enteric coated capsule that will carry penicillin to the small intestine before dis-integrating. The ideal coat-

food greatly increases the destruction of penicillin. When the drug was taken with a meal, no measurable amount was present in the blood at the end of 2 hours and only approximately 1% was excreted in the urine. When taken on an empty stomach, the amount excreted varied from 6.6 to 15.9%. On 1 experiment, it was noted that penicillin appeared in the urine 3 minutes after oral medication.

One group of subjects (Table 2) was given the drug dissolved in a saponified lanolin-

sesame oil base.⁸ Capsules were given containing 10,000 to 20,000 units in 0.5 to 1 gm. of the base. The subjects were allowed to have liquids only. An initial dose of 200,000 units was given followed by a second dose in 4 or 5 hours as indicated in the table. Blood samples were taken at hourly intervals for 8 hours and urine sample of 100,000 units was given initially and repeated at the end of 3 hours. Blood samples were taken at hourly intervals for 3 hours and, in 3 subjects, at the 6th hour. In 8 of the 10 subjects, blood levels ranging from 0.03 to 0.12 unit per cc. were still present at the end of 3 hours. In the subjects of this group in which satisfactory

TABLE 2.—BLOOD LEVELS OF PENICILLIN AFTER ORAL ADMINISTRATION OF 200,000 UNITS IN SAPONIFIED LANOLIN AND SESAME OIL EVERY 5 HOURS

Subject	Blood levels (Oxford units per cc. of blood)						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.
1. Ha.	0 03	0 03	0 03	0 03	0	0 03	0 06
2. Ga.	0 015	0 015	0 015	0 015	0	0 015	0 03
3. Di.	0 03	0 06	0 03	0 03	0 03	0 12	0 015
4. Ma.	0 24	0 06	0 03	0 12	0 015	0 12	0 015
5. Ma.		0 06	0 015	0 03			
6. Hal.	0 03	0 03	0 015	0 06			
7. Sm.	0 03	0 12	0 12				
8. Hi.	0 12						
Average	0 07	0 062	0 036	0 038	0 015	0 06	0 042

TABLE 3.—BLOOD LEVELS OF PENICILLIN AFTER ORAL ADMINISTRATION OF 100,000 UNITS IN ENTERIC COATED CAPSULES EVERY 3 HOURS

Subject	Blood levels (Oxford units per cc. of blood)			
	1 hr.	2 hrs.	3 hrs.	6 hrs.
1	0 06	0 12	0 12	0 06
2	0 24	0 24	0 06	0 06
3	0 48	0 12	0 06	0 48
4	0 24	0 12	0 12	
5			0	
6		0 06	0	
7		0	0 03	
8	0 24	0 06	0 06	
9	0 48	0 24	0 12	
10	0 48	0 48		
Average	0 24	0 16	0 069	

TABLE 4.—BLOOD LEVELS OF PENICILLIN AFTER ORAL ADMINISTRATION WITH 100,000 UNITS OF CALCIUM PENICILLIN IN ALUMINUM HYDROXIDE GEL

Subject	Blood levels (Oxford units per cc. of blood)			
	1 hr.	2 hrs.	3 hrs.	4 hrs.
K	0	0 06	0 015	0 015
L	0 03	0 015	0 015	0
R	0 12	0 06	0 03	0
F	0 12	0 03	0 015	0 03
W	0 24	0 12	0 03	0
G	0 03	0 03	0 06	
St	0 24	0 24		
Average	0 11	0 079	0 03	0 007

samples were collected for 12 to 24 hours. As indicated in the table, blood levels of 0.015 unit per cc. or above were maintained over an 8 hour period. The amount excreted in the urine varied from 3 to 20%.

A third group of subjects (Table 3) received the drug suspended in cocoa butter and placed in enteric coated capsules. A

urine studies were made, the percentage of excretion varied from 3 to 21%. As compared with the results of the first 3 hours with the lanolin-sesame emulsion, it is apparent that the enteric coating facilitated higher blood levels with half the penicillin dosage.

A fourth group of subjects (Table 4) was

given 100,000 units of the drug with 20 cc. aluminum hydroxide gel. Blood levels were followed for 4 hours and urine concentration for 6 to 8 hours. Subjects were given a soft diet low in protein and fat. As compared to the controls it is apparent that the amount of penicillin absorbed is greatly enhanced. Two subjects were given 25,000 units of penicillin in aluminum hydroxide gel prepared according to the method of Welch.¹⁰ This dosage was repeated every 2 hours for 4 doses. Blood and urine samples were taken at hourly intervals for 7 hours and urine collections were continued at intervals for 24 hours. No blood levels of penicillin were demonstrable by our method at any time and only traces appeared in the urine after the 9th hour.

Activated charcoal was found to remove over 99% of penicillin activity, and all but traces of the color and taste of an aqueous solution that was passed through it. On taking 3 gm. of the charcoal containing 99,000+ units of penicillin by mouth in gelatin capsules, however, no measurable amount was found in the blood and only traces appeared in the urine. It was concluded that either charcoal did not prevent destruction by acid or held the penicillin too firmly to allow absorption. To test this hypothesis, 100,000 units were passed through charcoal and assay of the filtrate showed that 99,730 units had been adsorbed. The charcoal was taken in gelatin capsules along with 20 cc. aluminum hydroxide gel. Again no measurable blood levels of penicillin were found and only traces appeared in the urine.

Discussion. It is apparent that various methods may be employed to protect penicillin from destruction by the acid of the gastric juice. The results from the experiments described indicate that the use of alkali or the use of an enteric coating plus a suspending medium give the most satisfactory protection. Gastric acidity and gastric emptying are variable factors even in the same individual. A suitable antacid should be one which can be given in adequate amounts to neutralize the maximum amount of acid expected without at

the same time producing a highly alkaline reaction in those with normally low acidity. Other desirable features are the ability to adsorb penicillin and the property of forming an adherent coating to the stomach and intestinal walls. A comparison of the efficiency of various antacids is being conducted.

Preliminary trials with some of the commonly used enteric coatings, especially phenyl salicylate, keratin and stearic acid, showed them to be unsatisfactory. In regard to phenyl salicylate as an enteric coating, it is well to keep in mind the admonition of Sollman⁸ that "a coating sufficiently heavy to be effective introduces large doses of salol." Examination of capsules coated by routine methods in a pharmacy revealed them to contain 0.5 gm. or more each. The maximum dose recommended is 2 gm. Fatal cases from phenyl salicylate administration³ add weight to Sollman's admonition. The use of an enteric coating that depends primarily on a time factor is undesirable because of the variable emptying time of the stomach and activity of the small intestine. The resin coating employed in the experiments disintegrated gradually in an acid medium, but slightly more rapidly in an alkaline medium. The time factor is apparently the dominant one with this coating.

The apparently firm adsorption of penicillin by activated charcoal makes this combination useless for oral administration. This observation is in agreement with the result of Henry and Henry⁶ who found that penicillin inhibits the adsorption of methylene blue by activated charcoal.

Summary. Penicillin has been given by the oral route with a number of vehicles. When given on an empty stomach in tap water, blood levels and urinary excretions are considerably greater than when the drug is given after a meal. Penicillin, when given in solution in saponified lanolin and sesame oil, shows enhanced and prolonged blood levels and increased urin-

ary excretion of the drug as compared to the controls with tap water.

The use of an enteric coating in addition to a suspending medium more than doubled the efficiency of absorption of penicillin as compared to a suspending media alone.

The use of aluminum hydroxide along with penicillin gave blood levels of penicillin less than those obtained with an enteric coating. No confirmation was found of the prolonged blood levels of penicillin reported for its use after adsorption by aluminum hydroxide gel.

Penicillin is too firmly adsorbed by activated charcoal to make this combination of value for oral administration.

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MEDICINE

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INFECTIOUS HEPATITIS

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THE disease entity or entities included under the term *infectious (non-spirochetal) hepatitis* are apparently caused by a filterable virus or group of such viruses which as yet have not been typed for identifying characteristics. The disease is characterized by non-suppurative hepatitis and a systemic response. Jaundice is usually an outstanding feature. In times of war epidemics may be extensive but generally the disease is sporadic. The incubation period approximates 3 to 4 weeks but under certain circumstances may be much longer.²³ The icteric phase of the disease runs a variable course of 3 to 6 or 7 weeks and is followed by complete clinical resolution after a somewhat prolonged convalescence. The term *infectious (non-spirochetal) hepatitis* does not include all types of *infectious hepatitis* not due to leptospiral organisms. Amebic hepatitis, yellow fever, and general infections, including syphilis, pneumococcal infection, malaria, tuberculosis, infectious mononucleosis, streptococcal disease and Oroya fever, are excluded, as are the clinical pictures resulting from hepatotoxic chemicals.

Many terms have been used to describe this disease. Sporadic cases have been called *catarrhal jaundice*, a term which very likely has included hepatic de-

generation from toxic chemicals when the latter are unknown and probably other types of disease as well. Epidemics have been traced to sporadic cases and the pictures cannot be distinguished. Even though some²² may still feel they are separate diseases, the problem cannot be settled until etiologic factors are settled. The term *catarrhal jaundice* developed with Virchow's idea that a catarrhal inflammation of the bile ducts was responsible for the picture. As late as in World War I the idea was still prevalent, and Hurst²⁶ believed that *catarrhal jaundice* and *infectious hepatitis* were different diseases. Other terms used include *infectious hepatitis*, *infective hepatitis*, *epidemic catarrhal jaundice*, *common infective hepatic jaundice*, *non-spirochetal infectious jaundice*, *campaign jaundice*, *camp jaundice*, or the equivalents in other languages, as *ictère contagieux*, *jaunisse de champs*, or *Kriegsicterus*.

ETIOLOGY. Recent work indicates that a filterable virus or group of viruses is responsible for the clinical picture. Characteristics suitable for classifying the agent are lacking. For many years the possibility of a living agent as the cause had been suspected, chiefly because of epidemic occurrence with seasonal peaks in

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the autumn and early winter, the rather constant incubation period, and the clinical course. Epidemics have been prone to appear in troop concentrations at such times as in the Napoleonic Wars, the American Civil War, the Spanish American War and both World Wars. In the recent war, numerically it proved to be one of the most important diseases affecting our armies. In the central Mediterranean area the disease reached great epidemic proportions. The importance of the disease in the armed forces has stimulated a great number of investigations which have clarified some of the factors in its etiology. Voegt⁶¹ appears to have been the first to transmit the disease to volunteers by the oral administration of duodenal juices and urine. Findlay and Willcox¹⁴ have caused typical icteric and subicteric cases to develop after oral ingestion of feces or of urine from spontaneous cases. Feces filtered through a Seitz filter proved infective in 1 experiment. Such experiments as these, as well as others with transmission through the subcutaneous and intravenous routes establish tentatively that a filterable virus is the probable cause. Blood and stools from patients with infectious hepatitis, taken especially in the pre-icteric stage and up to 2 days after appearance of jaundice, are most uniformly infective. As stated, the duodenal juice is infective in the same stages of the disease. The active agent besides being filterable will withstand 56° C. for ½ hour. It has not yet been identified by the electron microscope.

The natural means of transmission are not clear. The enteric route has long been under strong suspicion. In the Scandinavian countries epidemics have been found in pigs, and in patients an increased incidence was found in the rural areas.² Jaundice developed in pigs fed liver from jaundiced pigs and bile from human cases. In general, attempts to transmit the disease to animals have failed. MacCallum and Miles³⁴ have produced in rats on a protein deficient diet a liver disease by

inoculating the blood and feces from patients with infectious hepatitis but English observers have been unable to confirm transmission to pigs. Blumer⁷ in this country has noted jaundice following ingestion of pork. Excretion of the virus in the feces and the transmission experiments already cited with feeding of infected feces and duodenal juices strongly favor the oral route of spread. In 1 report of an epidemic in a camp (Neefe *et al.*⁴⁰) the agent was found in well water and was probably the same as that excreted in the feces of the camp patients. Swedish reports¹⁸ also indicate that the disease may be water-borne. Recently Murphy and associates³⁸ have presented epidemiologic evidence that the disease may be transmitted by milk. In an outbreak of 10 cases in Georgia the evidence, though circumstantial in character, pointed to a single milk supply as the source. Read and her associates⁵⁰ in Cleveland studied an outbreak in a medical fraternity and found affected primarily those who ate regularly at the fraternity dining table. The cases were not traceable to previous cases.

The above evidence does not exclude other possible routes of transmission. Droplet infection has figured prominently in suggestions of natural spread. It is not clearly established but is thought by some to be a likely mode of transmission. The disease has been transmitted to volunteers³³ with a nasal spray of nasopharyngeal washings from subjects with the disease in the pre-icteric stage or within 24 hours after developing jaundice. In some such studies infection has occurred in 30 to 50 days.¹² Thus natural infection very likely occurs either by droplet infection through the upper respiratory tract or by food through the gastro-intestinal tract.

Mention must be made here of the clinical entity called *homologous serum jaundice*. This picture at times follows the injection of blood or its products, usually in 6 weeks to 6 months. It has occurred following whole blood transfusion, dried, frozen, or

fresh plasma and serum. It is the type seen in subjects following inoculation with certain batches (icterogenic batches) of yellow fever vaccine containing human serum. This symptom complex is not yellow fever.⁵⁶ There is a difference in symptoms and furthermore the virus of yellow fever has not been isolated from these patients. The immune titer for yellow fever is constant as it is in those without jaundice. Also patients already immune to yellow fever have developed homologous serum jaundice and in some of those in whom immunity to yellow fever has not developed because of defective vaccine the picture has appeared. Additional evidence rests in the development of the symptom complex after use of human serum without yellow fever vaccine, especially after the use of measles and mumps convalescent serum. It has also been noted in diabetic and venereal disease clinics in which syringes, contaminated with serum of a previous patient, have been used on other patients without heat sterilization. With contaminated plasma as little as 0.01 cc. has been known to produce the disease. Thus, as well as in contaminated syringes with small amounts of serum, pooled plasma in which someone's serum containing the agent has been added, though highly diluted by the other sera, can produce the disease. In casualties returned to the United States in the past war the frequency of the picture has been as high as 2 to 3%.

The question immediately arises whether homologous serum jaundice is identical with infectious hepatitis, or whether it is a separate disease process. This will not be settled until the causative agent or agents of each are more clearly identified. The clinical manifestations are strikingly the same, but cross-immunization apparently does not occur. Patients are known^{57a} to develop infectious hepatitis after having had postvaccinal hepatitis (yellow fever vaccine) a year before. Studies by Neeffe, Stokes and Gellis⁵² indicate that volunteers recovered from homologous serum jaundice and then inoc-

ulated with material known to contain a causative agent of infectious hepatitis developed hepatitis; controls, including reinoculations with material of homologous serum jaundice, were negative. Havens⁵³ has obtained similar results. The modes of transmission are, of course, different. Also homologous serum jaundice is seldom connected with secondary cases, unlike infectious hepatitis. The most striking difference in the 2 groups is the extremely long incubation period of homologous serum jaundice. In yellow fever vaccine cases the period has been 9 to 23 weeks (Turner *et al.*⁵⁹). In contaminated syringe cases⁵⁴ this period has averaged about 3 to 4 months (Marshall⁵⁵). Experimentally induced attacks produced by inoculations of the original dried pooled human serum from a blood bank which had been used in an icterogenic lot of yellow fever vaccine have shown incubation periods between 59 and 129 days (MacCallum and Bauer⁵²). Serum from volunteers who have developed the short incubation form after oral administration produces the short incubation form in other volunteers, whether given orally or parenterally. The agent has survived a temperature of 56° C. for 1 hour and storage in a dry state at 0° C. for many months as well as in a liquid state for a considerable time. The same characteristics hold for the agents of infectious hepatitis.

The difference between the incubation period of naturally occurring infectious hepatitis (about 4 weeks) and that of homologous serum jaundice (3 to 4 months) has been a strong argument that these disease states are separate entities. The lack of facility in experimental transmission by oral administration of feces of homologous serum jaundice,⁵² as compared to the ease of transmission of infectious hepatitis, is another point against their unity. Another point which suggests a difference is the failure of homologous serum jaundice to spread from patient to patient and the frequent spread in infectious hepatitis. In 1 study (Neeffe *et al.*⁵⁴), injection of homologous serum

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products into volunteers showed incubation periods from inoculation to the first appearance of jaundice of from 73 to 110 days although first manifestations of hepatitis were noted within 12 to 50 days in 7 subjects and in from 12 to 35 days in 6 of these. Jaundice appeared late in the hepatic picture. This short incubation period directly concerns the relationship of the disease to infectious hepatitis. Possibly immune bodies in the serum could lengthen the incubation period by temporary protection. Treatment of water contaminated with an agent producing infectious hepatitis is known to have resulted in a decrease in the concentration and possibly the virulence of the agent, so that some modification of the disease may be produced. Evidence suggests that such changes in virulence or concentration may be associated with a prolongation of the incubation period (Neefe *et al.*⁴¹).

In general then, from the above remarks, it can be seen that the chief differences in the 2 pictures rest in the incubation periods, and epidemiology, the routes of infection, immunity experiments, and, to some degree, in the severity of the clinical picture and mortality figures.

Parenthetically, it may be stated here that certain instances of so-called post-arsphenamine jaundice are not toxic arsenical reactions but homologous serum jaundice. Clinical pictures are indistinguishable and liver biopsies have shown identical changes. As already stated, transmission of the disease has occurred in venereal disease clinics in which syringes have been sterilized by washing with water and alcohol rather than by heat. Bigger⁵ has shown that staphylococci mixed with blood drawn into a syringe are not completely removed or killed by this technic. The agent, not destroyed by this means of sterilization, may be injected into the next individual upon whom the syringe is used.³¹ In addition there is suggestive evidence (Findlay and Willcox¹⁴) that concurrent injections of arsenicals may increase the liability to

the disease. The same type of transmission has been reported in a diabetic clinic.¹⁰

PATHOLOGIC ANATOMY. The pathologic picture of infectious hepatitis was long misunderstood, primarily because of paucity of postmortem material. In 1855 Bamberger suggested that swelling of the ostium of the common bile duct produced jaundice through obstruction. In 1865 Virchow described a case in which the ostium of the common bile duct was plugged with mucus and he coined the term *catarrhal jaundice*. The traditional concept that a catarrh of the duodenum involved the ampulla of Vater with a mucus plug was supported by clinical evidences of nausea, dyspepsia and other gastro-intestinal symptoms and was not controverted by autopsies, which were infrequent. Many years later Van Rooyen and Gordon⁶⁰ carried out intubation studies and showed there is no evidence of duodeno-biliary catarrh. According to Lichtman,^{28a} A. Stokes' view, that mechanical obstruction was not the basis of the picture, was first postulated by him in 1839, long before Virchow's prestige established his views, which in turn were supported by reports of a small number of cases with inflammation of the bile ducts. It was not until the period of World War I that hepatitis was generally conceded to be the cause of the picture. At that time Eppinger¹¹ found, in the livers of soldiers with this disease who had died as the result of trauma, hepatitis without obstruction. Similarly in the Gallipoli campaign necrosis of parenchymatous cells was found under the same conditions. Still later,^{9,51} biopsy in various stages of the disease showed a picture characterized by acute necrosis and autolysis, beginning at the center of the lobules, disorganization of the liver cell columns, and leukocytic and especially histiocytic infiltrations into the portal zones. At this time the widespread damage of the liver is so extensive that one wonders how recovery takes place. Reticular framework is not lost and rapid recovery usually ensues. Patients erroneously operat-

ed upon show in their livers swelling with tissues stained green by bile pigments. Biopsies reveal cloudy swelling. With recovery the picture returns to normal. Recent studies (Lucke²⁰⁰) of patients who died of accidents at variable intervals after infectious hepatitis have confirmed these views by proving healing with only slight residual traces of liver injury not indicative of persistent or progressive damage.

Severe attacks of infectious hepatitis leading to death have supplied material for pathologic study. In the liver the picture resembles that of acute yellow atrophy. This form of yellow atrophy differs from that caused by hepatotoxic chemicals (Lucke²⁰⁰).

Recent studies of livers in homologous serum jaundice have shown similar pictures with necrosis, atrophy, and varying degrees of regeneration. Councilman bodies, as found in yellow fever, have also been noted (Hayman²⁴).

Lesions in organs other than the liver also occur in infectious hepatitis. The spleen is enlarged in the majority of instances due to cellular proliferation and, in later stages, to congestion. Lymphadenopathy appears. The kidneys may show the characteristics of the lower nephron syndrome, a cholemic nephrosis. In late stages hemorrhages are common, especially in the lungs and gastro-intestinal tract as well as in other tissues such as the heart, mediastinum and pleura. Electrocardiographic changes have been described (Louis²²), but the author has not been able to see the report. The bone marrow shows moderate hyperplasia. The brain may show non-specific degeneration of ganglion cells and at times a mild meningoencephalitis is seen. Ascites is frequent.

SYMPTOMS. The onset of symptoms follows an incubation period averaging 3 to 4 weeks in the naturally occurring disease where knowledge of contacts has permitted measurement; in homologous serum jaundice the incubation period has averaged approximately 4 months. (See

section on Etiology.) In sporadic cases no relationship to other cases may be established.⁴ The clinical symptoms in naturally occurring and homologous serum jaundice are so similar that one description will suffice for both.

Symptoms may not occur at all. In epidemics symptoms without icterus and instances without symptoms but with evidences of silent hepatitis have been found.¹⁸ Pollock^{47c} has reported instances in small children which he believes to be symptomless attacks of infectious hepatitis. Final etiologic proof is lacking. When findings do occur the course and severity are variable. Three stages are, in general, recognized. A *pre-icteric* stage of prodromal symptoms before jaundice and hepatomegaly appear leads to an *icteric* stage in which hepatomegaly and jaundice are present. The icteric stage is followed by a stage termed *convalescent*, when the disease takes its usual course, or *final* in those few instances in which a fatal termination is imminent.

Pre-icteric Stage. In several large series^{3,20a} this stage has been definite in 83% of the cases and lasts generally about 5 days. All grades of severity are seen, from mild to extremely prostrating symptoms. Anorexia may be the chief symptom. Epigastric distress, nausea, vomiting and weakness may appear. Abdominal distention and cramps also develop. Occasionally malaise or urticaria may be seen. Headache, constipation or diarrhea, and joint pains sometimes develop. In Bercovitz and Knoch's group, 13 of 54 patients had prior symptoms of upper respiratory infection. In Havens' series of 200 patients in the Middle East and in Bercovitz and Knoch's and Finks and Blumberg's groups, the frequencies of the symptoms are shown in the table.

At times the patient may complain of nothing except perhaps a feeling of being below par for a few days before jaundice is noted. His attention may be drawn to the jaundice by friends. In general, the type of onset varies greatly within the same group of cases. Most usual is the

insidious onset with anorexia, epigastric distress and dark urine. At times a more acute onset is more frequent. In 1 outbreak in the Lebanese Republic,⁶⁴ 70% of the patients had onset with chills and moderate fever which subsided in 4 to 5 days. In such instances the temperature usually drops with onset of jaundice, or 24 to 48 hours before. In a Canadian epidemic⁵⁴ the average patient had sudden onset with fever, anorexia, nausea and abdominal pain. Fever in such instances may go to 104° F. or more. Headache may be severe and vertigo and generalized body pains, particularly in the arms, neck and legs, occur. About one-half the patients in Havens' group had sudden onset with chills or chilliness and remittent temperature to 102° F. More usually fever, if present, is low grade. Finks and

ed and the hippuric acid test in Finks and Blumberg's¹⁵ experience has been helpful.

Icteric Stage. After the onset of jaundice, or just before it appears, the temperature may become slightly elevated but not to the previous peak. The gastrointestinal symptoms persist and are likely to be accentuated. Anorexia may become severe and nausea and vomiting, if not present before, may develop. Distention and heaviness in the epigastrium are common. Right-sided abdominal pain may be accompanied by tenderness and simulate acute abdominal disease. The depth of jaundice varies greatly; it may be light or deep and the average duration is about 3 to 4 weeks, although periods of 5 to 72 days, or even longer, are known. It usually reaches its height before the 10th day and then begins to recede. In general,

	Havens, percent.	Bercovitz and Knoch, percent.	Finks and Blumberg, percent.
Anorexia	82	88.8	87
Nausea	82	87.0	84
Weakness	75		
Epigastric distress	42		
Abdominal discomfort	37.0	
Vomiting	27.7	51
Generalized malaise	25.9	34
Diarrhea	11.0	33
Constipation	5.5	22

Blumberg¹⁵ stress the occasional occurrence of fever in postvaccinal hepatitis and its frequent occurrence in their group. Similarly in the postvaccinal group incidence of dermatitis was high. Lymphadenopathy was found frequently in the naturally occurring group and is often not mentioned in the postvaccinal cases.

The interval from onset of symptoms to development of jaundice varies from 1 to 18 days. In approximately 75% jaundice appears by the 6th day. The average is often given as 5 days. Hepatitis may occur without the development of jaundice and the diagnosis is likely to be missed except in an epidemic. The liver may be enlarged and tender and lymphadenopathy may occur. Laboratory data may not be helpful and icterus indices may be normal as may the van den Bergh reaction. Urinary urobilinogen is increas-

the symptoms of this period correlate with the icterus. Itching is not common with the jaundice. The stools may become light in color, often for a period averaging about 8 days, but are seldom typically clay colored. Lymphadenopathy has been found in as high as 81% of naturally occurring cases and at times may be a striking feature. The nodes varied from the size of a small pea to that of an English walnut. The size and consistency vary. Early in the disease they may be soft, non-tender and discrete. Later they become firm, smaller and shotlike.¹⁵

When jaundice fails to appear the clinical picture, though otherwise a typical one, is likely to be milder than usual and of shorter duration.

Final Stage. This stage occurs in those cases in which in the icteric stage events take a turn from the usual benign course

to a serious severe course resulting in death. In elderly patients and in pregnancy the disease is sometimes prolonged and the symptoms may be severe. It is apparently more apt to occur when the individual is in a state of malnutrition or exhaustion when infected. This stage is so infrequent in occurrence that it does not constitute a part of the average case. Here jaundice continues and even increases. Acute liver failure ensues. Nervous symptoms, lethargy or coma alternating with restlessness, excitement, delirium, muscle weakness, scanning speech and exaggerated reflexes usher in this phase which usually occurs before the 20th day of the disease, and most often within the first 10 days.

Convalescent Stage. This stage, rather than the final stage described above, usually follows the icteric period. In this period exercise is known to cause recurrence of symptoms and signs and graduated exercises are used as final criteria of clinical recovery. In about 10% of Barker's³ cases, recovery has not taken place within a 3 months period.

PHYSICAL FINDINGS. In the pre-icteric stage physical findings are not outstanding. Tenderness may develop in the right upper quadrant of the abdomen and hepatic enlargement is also usually present before jaundice appears. Splenomegaly and lymphadenopathy may appear in this stage. Lymphadenopathy has already been discussed. The outstanding finding of the icteric stage is, of course, jaundice. The liver, often already enlarged as stated above, increases in size usually to 3 or 4 finger breadths below the costal margin. It is reported as palpable in anywhere from 20 to over 70% of the cases. Splenic enlargement appears in 10 to 25% of the cases. In some epidemics⁴ splenomegaly has been reported in one-third of the cases. Tenderness accompanies the hepatic enlargement in two-thirds or more of the cases and may be present when the liver cannot be felt. Hepatomegaly usually correlates with the progress of the disease, reaching

its height, on the average, in 16 to 17 days and then starting to recede. Bradycardia is uncommon.

Changes in the skin are sometimes seen. These include erythema nodosum, urticaria, petechiae and purpuric areas. Bleeding gums are also seen. In severe cases with a prolonged course, spider angiomas may appear. The final stage is characterized by an increase in icterus, coma and the neurologic findings stated above, at times a shrinkage in the size of the liver, and sometimes hemorrhagic phenomena such as epistaxis, hematemesis and hematuria.

The convalescent stage is characterized by a recession of the physical findings. Hepatomegaly may appear following exercise and occasionally jaundice may reappear for the same reason.

LABORATORY DATA. The laboratory data vary with the stage of the disease. In the *pre-icteric stage* measures of bilirubinemia, such as the icterus index and van den Bergh test, are normal except in the period immediately preceding the appearance of jaundice. Bilirubinuria occurs 1 to 3 days before clinical jaundice appears. One-half to three-quarters of the cases (Barker³) show a positive cephalin cholesterol flocculation test in this phase. At times the bromsulfalein and alkaline phosphatase tests may be abnormal. In this stage the blood sedimentation rate is not markedly elevated, a point held helpful by some in the differentiation of other states in which fever is associated with a rapid sedimentation rate.

In the *icteric stage* the icterus index is, of course, elevated. Figures to 100 or 150 are not uncommon and readings have been found over 300. In the 2nd to 3rd week, often about the 10th day, the icterus index starts down and usually is normal by the 5th or 6th week. The *van den Bergh reaction* is at first indirectly positive, but may change to a direct reaction as the icterus index elevates. *Urobilinogen* is found in abnormal range as the icterus index rises. The urine shows bile, as stated above, in the terminal phase of

the pre-icteric stage. Albuminuria of mild degree is not uncommon. Stools become light in color during the early part of the icteric stage and may persist as such until the height of the disease. Although many patients describe stools as clay colored, seldom are such stools actually present. The *prothrombin time* may be prolonged and, with severe liver damage, is not corrected by vitamin K therapy. As in the pre-icteric stage, the *cephalin cholesterol flocculation test* is positive in a high percentage of cases. The figure exceeds 90%. It is almost universally positive in patients with acute symptoms. It becomes negative in 3 to 4 weeks but at times may persist for 3 or 4 months. Other evidences of disturbed hepatic function may appear. These include changes in *blood proteins*, as reflected in actual measurements or in the *colloidal gold test*, depression in *serum amylase* followed by an elevation and retention of bromsulfalein. *Blood cholesterol* levels may be elevated. In general, the *glucose tolerance curve* is not markedly changed. In anicteric cases dye retention and the cephalin cholesterol flocculation test are most important, as are the other tests (aside from those for bilirubin) mentioned above.

The blood sedimentation rate is not markedly elevated. The white blood count is, in general, not elevated. Moderate leukopenia is often present, often starting in the pre-icteric stage. Atypical lymphocytes, often to 5 to 20%, similar in type to those seen in infectious mononucleosis, are seen.³⁶ Heterophilic antibodies are absent. In about 5% of the cases the white blood count exceeds 10,000.

Final Stage. In the few cases reaching the stage of acute yellow atrophy the white blood count is more likely to be elevated, even to 25,000. The usual findings of acute yellow atrophy are seen.

Convalescent Stage. This stage is heralded from the laboratory standpoint by a reduction in the blood bilirubin to below 2 mg. and the van den Bergh becomes indirect. The icterus index drops below

20 units. Likewise, the bromsulfalein retention is reduced.

DIAGNOSIS. The greatest difficulty in diagnosis occurs in the pre-icteric stage, although with the diagnosis in mind one may suspect its presence when anorexia, hepatic enlargement and hepatic tenderness appear. Evidence to diagnose the disease in the pre-icteric stage may be meager. This is particularly true in those instances with fever. Aside from malaria, other conditions to be considered in the differential diagnosis in this period include upper respiratory infections, atypical pneumonia, infectious mononucleosis, enteritis and influenza. In tropical areas besides malaria, sandfly fever and dysentery may be confusing. In an epidemic suspicion of the diagnosis is high and the laboratory findings already mentioned may be helpful in diagnosis. Pollock^{47a} has found in the early stages a qualitative change in serum bilirubin with the appearance of small quantities of bilirubin in the urine. He found that the pre-icteric stage of the disease is characterized by an abnormal retention of bromsulfalein, the excretion of small quantities of bilirubin in the urine and the development of an abnormal direct van den Bergh reaction. The serum bilirubin and urine urobilin levels do not usually rise above normal limits until shortly before jaundice appears.

Hallock and Head¹⁹ found that in troop concentrations the demonstration of pathologic amounts of urobilinogen in the urine was very helpful in 9 out of 10 patients in this stage. Gellis and Stokes¹⁶ have shown that the methylene blue test for urobilinuria is positive in the pre-icteric stage. The cephalin flocculation test may also be positive in the pre-icteric stage. When fever is the problem in differential diagnosis Wood⁶³ found the sedimentation rate below 10 mm. in 85% of the cases, a point he felt useful in differentiating malaria. Repeated examinations for hepatomegaly and its appearance, together with tenderness, are most helpful. There is no specific test

for the disease, and other forms of hepatitis may be confused with it at this time. The diagnosis will not become certain until jaundice appears. In cases in which icterus does not develop the characteristic history and physical findings along with laboratory data indicated suggest the diagnosis, especially in an epidemic or when the examiner keeps in mind the fact that such an entity exists.

In the icteric stage diagnosis rests upon the elimination of infections, such as pneumococcic and streptococcic septicemia, malaria and syphilis, in which hepatitis is more or less incidental to systemic infection; other types of hepatitis in which the hepatic findings are the outstanding clinical features, such as yellow fever, leptospirosis, occasionally amebic hepatitis with jaundice, and infectious mononucleosis; and, finally, degenerative changes in the liver resulting from hepatotoxic chemicals, such as arsenic, carbon tetrachloride and cinchophen. If jaundice does not appear in infectious hepatitis, hepatomegaly with tenderness will still demand differentiation of the above states.

In the convalescent stage diagnosis will rest on history, possible persistence of hepatic dysfunction, its recognition by laboratory means, the ruling out of similar states by such evidence as negative serologic reactions for leptospirosis and infectious mononucleosis, along with possible reactivation of the clinical picture by exercise.

COMPLICATIONS. In general, complications of infectious hepatitis are not common. Some of the so-called complications are actually less common clinical manifestations of the disease itself. This is true of nervous symptoms, such as numbness and weakness of the limbs, and meningeal symptoms early in the disease. Hemorrhage is in the same category. Likewise, in the final stage, described above, the picture of acute yellow atrophy appears, with mental excitement, convulsions and coma. Pulmonary, cardiac and gastro-intestinal symptoms are well established. As sequelæ to the disease, nephri-

tis and cerebral hemorrhage are known. Relapse of the clinical picture occurs, particularly when exercise has been permitted too early in the convalescent stage. Commonly the convalescent stage is prolonged, especially if prostration has been marked. In some series,³ 5 to 10% of the patients are not clinically well at the end of 3 months.

Recurrence of infectious hepatitis in the same patient is considered uncommon but does happen. In 1 series¹³ only 4 individuals of 2614 had had more than 1 episode of jaundice. Nelson⁴⁵ reported 2 patients in whom 5 episodes of jaundice occurred and in whom he interpreted the episodes as recurrent infectious hepatitis. Inability to demonstrate the etiologic agent always throws doubt on the veracity of such reports despite elaborate means for differential diagnosis.

Stokes, Owen and Holmes⁶⁸ have found as have others that neurologic findings have been protean. They have found 4 main groups. First is the picture occurring in the fatal case with drowsiness, apathy, stupor, or maniacal symptoms, coma, and convulsions, symptoms found in all types of acute hepatic necrosis. Second occurs the symptom complex of generalized or localized muscular rigidity with increased tendon reflexes and at times choreiform movements. Striatal and pyramidal signs are outstanding. Third large focal hemorrhages may occur in nervous tissue and at times produce localizing signs. Finally, peripheral neuritis is described.

In epidemics in the Armed Forces¹ there have been men who, despite complete recovery as attested by laboratory data, continue to show fatigue, right upper quadrant discomfort, digestive disturbances, especially fat intolerance, malnutrition and emotional instability. Despite the lack of evidence of an organic lesion to account for these symptoms, the picture has shown a constant pattern.

Chronic, persistent, hepatic damage has been shown in a small number of cases. This may be manifested as a latent hepatic

itis^{1,27,28} with persistent subclinical icterus and decrease in the bilirubin excretion test, in some patients years after attacks of infective jaundice. Symptoms, chiefly chronic gastro-intestinal complaints, have occurred, but often the disease is entirely latent. Bloomfield⁶ believes that some such cases may gradually progress until eventually, perhaps after years, the clinical picture of hepatic cirrhosis appears. Others⁴⁹ find a history of jaundice in 6.5% of patients with cirrhosis and feel that possibly permanent structural liver damage may occur following such states. Although some discussion has been given these residual states, it must be remembered that the usual finding is complete recovery.

PROGNOSIS. In general, the course of infectious hepatitis is mild and the prognosis excellent. The mortality rate is extremely low, usually being given as less than 0.5%. The natural history of the disease depends to a considerable extent upon the condition of the liver prior to the onset of the illness and upon factors which affect its capacity for regeneration during the course of the disease. Thus, in elderly patients the course is more apt to be long and serious. Sequelæ are more likely. Those with past liver damage and chronic alcoholism do not do well. In some outbreaks mortality has been higher in groups on less adequate diets.⁶² In pregnancy the picture is often a severe one and may prove fatal. The development of the final stage or phase, with the picture of acute yellow atrophy, is thought to be more frequent when treatment has been neglected early in the disease, especially the neglect of rest.³ Hemorrhage, especially with prolonged prothrombin time not responsive to vitamin K therapy is a serious sign.

TREATMENT. Early diagnosis and institution of treatment, especially in the pre-icteric stage, are thought to be most important. The foundations of successful treatment are rest and adequate diet. Army experiences with homologous serum jaundice (Turner⁵⁹) and naturally occur-

ring infectious hepatitis (Barker³) indicate the importance of rest. Patients who continue to work early in the disease do badly. Early bed rest decreases the severity of the disease and shortens the clinical and convalescent periods. Too early resumption of activity may result in recurrence, increased hepatomegaly and a return of symptoms. The same is true in patients in whom intercurrent infection of the respiratory tract occurs (Finks¹⁵).

Dietary therapy is most important. Generally it is stated that a high protein, high carbohydrate, low fat diet enhances the results achieved by rest. There is at times some dislike for excessively high protein (200 gm.) diets, and where a free choice of diet has been permitted patients usually prefer a diet of normal proportions. Under any circumstances the protein intake should not be low, and the carbohydrate intake should be high (400 gm.). Fat should be restricted. Anorexia may make for difficulty in intake of the proper diet. Frequent feedings are often helpful under these circumstances, but if nausea and vomiting make intake difficult intravenous glucose, 5 to 10%, is necessary. With protracted nausea and vomiting plasma or whole blood transfusion may be necessary to bolster protein intake. Two units of plasma a day will accomplish this end (Barker³).

From experimental data on liver protective diets, dietary supplements, especially multivitamin preparations, would seem proper therapy. Certainly with hemorrhagic phenomena associated with reduced plasma prothrombin levels, parenteral vitamin K in 2 mg. doses is in order. It and vitamin B₁ in excess have been thought harmful by some. The latter may be used in doses of 3 mg. daily by mouth. Adequate intake of other vitamins is indicated but their use in excess is not indicated. Alcohol is contraindicated. The known prophylactic value of methionine and choline in liver disease suggests their use in therapy. In general, they have not been shown to affect the

clinical course measurably.^{25,55,59,62} Doses of methionine have been 5 gm. daily.

Pollock's^{47b} studies of liver function in infectious hepatitis were gauged by the hippuric acid synthesis tests and indicate that the ability to synthesize hippuric acid bore some relation to the severity of the attack and showed improvement from the time of admission to the hospital, even though the icterus index and jaundice increased. He stated that it is probable that maximum liver damage in this disease most commonly occurs before the patient is admitted to the hospital. Specific treatment is, therefore, not likely to have any striking success unless begun in the pre-icteric stage.

Symptomatic and supportive therapy is applied as indicated. Morphine and barbiturates are to be used with caution because of the enhancement of their action in the presence of liver disease. Atropine or belladonna is helpful for abdominal cramps.

The criteria of Barker, Capps and Allen³ as to when the patient may be allowed out of bed include at least 3 weeks of bed rest, normal liver size, or, if palpable, lack of tenderness, absence of symptoms, especially lassitude and anorexia, bilirubin levels normal for a week or, if elevated, under 2 mg. per 100 cc., or icterus index under 16, absence of a prompt direct qualitative van den Bergh reaction. They think it desirable for the bromsulfalein dye retention to be under 10% in 1 hour and better under 6%. After the patient is ambulatory, he should be examined for increased tenderness and enlargement of the liver which, if present, indicate further bed rest.

PREVENTION. The preventive aspects of infective hepatitis are not yet on sound practical grounds, for the epidemiology is not well established. Existing evidence that the patient is infectious in the pre-icteric stage also makes the problem of control difficult. Evidence that droplet infection and spread through intestinal excreta are important has already been discussed. Water-borne virus has been

demonstrated (Neefe *et al.*⁴⁰). Hence, measures to combat both of these routes of spread are in order. There is evidence that the complete inactivation of the causative agent in drinking water may require further modification of methods used for water disinfection (Neefe *et al.*⁴¹). No prophylactic vaccine has been developed. There is some promise in the use of immune globulin in prevention of the disease when epidemics exist (Stokes and Neefe⁵⁷). Results thus far justify its trial under such circumstances; in some groups the routine use of 10 cc. on admission of men with previous transfusions is now being tried. The results have not yet been published. Effectiveness of immune globulin in prophylaxis has been confirmed in 2 field studies in the Mediterranean theatre (Gellis *et al.*^{17a}) which suggest that the globulin confers a period of passive immunity of at least 6 to 8 weeks. Its use has also been confirmed in at least 2 institutional outbreaks in the United States. In 1, the case rate for jaundice in the controls was about 10 times that noted among those inoculated. It was effective when given in the incubation period, preferably earlier than 6 days before the onset of symptoms. In an outbreak in a home for children (Havens and Paul⁵¹) striking differences in occurrence of jaundice in inoculated and uninoculated groups were noted. Only 2 of 97 inoculated children developed jaundice and these appeared within 6 days of the administration of the gamma globulin. The long incubation period of the disease lends itself well to prophylactic injections. Doses used have varied from 0.06 or 0.08 cc. to 0.12 or 0.15 cc. per pound of body weight intramuscularly. Apparently (Gellis *et al.*^{17b}) once the initial symptoms appear globulin is not helpful.

The prevention of homologous serum jaundice has been accomplished in part by the removal of human serum from the content of vaccines. In syringe transmitted hepatitis prevention can be accomplished by sterilization by dry heat (160° C. for 1 hour) of all syringes and

apparatus used for parenteral injections. Thorough physical cleansing of syringes is essential. Transmission by transfusion from donors before onset of symptoms (Murphy³⁷) might be difficult to control (Snell *et al.*⁵⁵) but certainly prospective donors with suggestive symptoms, with a history of hepatitis within the past year, with exposure to the disease within 3 months, or with transfusions themselves within the past 6 to 8 months should not be used. The possibility of using a donor when he is in the incubation period or subclinical stage is evident, especially in

epidemics. The virus circulates in the blood probably for some weeks before jaundice appears. The possibility of "healthy carriers" has been brought out (Rappaport⁴⁸). Recently a report has indicated that ultraviolet irradiation (Oliphant and Hollaender⁴⁶) of serum containing the etiologic agent of homologous serum jaundice appears to inactivate it. This suggests that ultraviolet irradiation might be useful as a routine procedure in processing potentially icterogenic human serum or plasma.

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NEUROLOGY AND PSYCHIATRY

UNDER THE CHARGE OF

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SCHIZOPHRENIA

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PRESENT day concepts of schizophrenia are largely based upon the formulations of Kraepelin, Bleuler and Meyer. Kraepelin³⁴ brought together a group of mental disorders, previously considered diverse in character, which he labeled "dementia præcox." The common features of these disorders comprised: (1) a lack of interest and emotional reactivity, and (2) a weakness of judgment or evaluation without impairment of memory. Kraepelin's dementia præcox was subdivided into the 4 types which are still commonly employed in psychiatric diagnosis: (1) simple dementia, characterized by progressive withdrawal and lack of interests; (2) hebephrenia, characterized by looseness of thought, silliness, and poorly fixed hallucinations and delusions; (3) catatonia, characterized by the presence of fixed postures and waxy flexibility; and (4) paranoid forms in which persecutory delusions predominate. Kraepelin assumed that dementia præcox was a degenerative disease of the brain or a metabolic disturbance and that the prognosis was uniformly poor.

Bleuler introduced the term "schizophrenia" which was based on his recognition of the absence of a true dementia and his concept of the "splitting of the psyche."⁵ The "basic symptoms" of schizophrenia, as outlined by Bleuler, were: (1) disturbances of association of ideas leading to incomprehensible, incoherent productions; (2) disturbances in affectivity with

emotional indifference or unnatural expression of emotion; (3) "schizophrenic ambivalence" in which mutually exclusive ideas exist side by side; and (4) lack of memory impairment.

Meyer,⁴¹ unlike Kraepelin and Bleuler, was not convinced by the evidence in favor of the organic origin of the disorder. He emphasized the clinical picture not as a disease entity, but rather as a type of reaction in certain personalities as a result of a progressive difficulty in adaptation. He noted that no true dementia or deterioration as seen in organic brain disease was present but that the accumulation of faulty habits of reaction led to a "habit deterioration." By the schizophrenic reaction type (or parergasia, as Meyer preferred to call it) was meant "those disturbances developing in particular constitutional types and expressed in vague, autistic fancy, projections, passivity, paranoid systemization, formal disorders of language and behavior, incongruity of affect and motility disturbances." The attempt was made "to avoid any fatalistic concept, recognizing deterioration as an end-stage of certain cases only, but preferring to focus on factors of modifiability and potential re-synthesis."

CLASSIFICATION. Most recent writers recognize the futility of attempting to divide schizophrenic disorders into sharply demarcated groups. Kraepelin's classification has continued to be used in spite of the difficulty in forcing many cases

into 1 of his 4 types. While noting the impossibility of making rigid categories and recognizing the frequency with which a patient will shift from one pattern to another, Terry and Rennie⁵⁰ have nevertheless suggested a number of clinical reaction patterns which they felt could be profitably used in the study of schizophrenia: Group I. Aggressive reaction, associated with paranoid trends or hypochondriacal delusions. Group II. Conversion reaction, with fantastic experiences of rebirth, spiritual missions, and self-punishment or self-sacrifice. Group III. Passivity reaction, with obsessive-compulsive ritualistic behavior or with periods of stupor sometimes alternating with periods of excitement. Group IV, Delirious reaction, with episodes of odd overactivity with delirious features suggesting the influence of a toxic condition. Group V. Affective reaction, with emotional disorders, especially depression, either at the onset or occurring in cyclic form. Group VI. Defeatist reaction, with incongruous day-dreams and delusions, empty and vague thinking, and slovenly personal and social habits.

Hanfmann and Kasanin,²⁴ in a study of conceptional thinking in schizophrenia, found that their cases fell into the following 6 groups: (1) "Neurotic" schizophrenic cases, in which the schizophrenic reaction comes only as a last resort, when neurotic defenses (obsessive or hypochondriacal neuroses) fail. (2) Acute episodic psychoses with marked overactivity, extreme dramatization of conflicts in a setting of fairly good contact with reality, and a tendency toward clear intervals. (3) Cases with a paranoid tinge with mild ideas of reference and occasional hallucinations, frequently associated with a history of alcoholism and homosexual conflicts. (4) Cases of "primary thought disorder" in which there is a striking involvement of intellectual functions with marked incoherence and irrelevance. (5) "Cases showing marked dissociation with extensive compensatory fantastic elaboration with primitive archaic ideas of omnipo-

tence coming to the surface, with the feeling of a certain mission in life that has to be performed, and with a variable regard for the ordinary realities of life." (6) Old cases of schizophrenia with ideas of reference and delusions of persecution associated with little emotional reaction to these ideas.

ETIOLOGY. No general agreement has been reached as to either the homogeneity or the etiology of the schizophrenic reaction. The trend in this country is to stress psychogenesis, although heredity and constitution, neuropathologic changes, and disturbances in biochemistry and physiology are still actively discussed as pathogenic features.

Sullivan,⁴⁹ for one, has questioned the unity of these disorders. He expresses the belief that 2 etiologically different reactions are included. One is characterized by an insidious development and a poor prognosis with signs and symptoms pointing to an organic degenerative disease. Sullivan is content to retain the Kraepelinian term, dementia praecox, for this group. He restricts the term schizophrenia to cases which he feels are primarily a "disorder of living" and without organic substrate. "The person concerned becomes schizophrenic as one episode in his career among others, for situational reasons, more or less abruptly. He may have had months or years of maladjustive living . . ." These patients are capable of recovery.

Meduna and McCulloch⁴⁰ have pursued a different course in dividing the schizophrenic disorders, largely neglecting psychogenic considerations. They recognize a "true" schizophrenia in Bleuler's sense with: (1) a disturbance of associations or weakness of judgment; (2) loss of interest and effect; and (3) lack of defect in perception, orientation and memory (sensorium). The onset in these cases is said to be insidious and the outcome characterized by progressive impairment of the mental and emotional life. Another group, labeled by Meduna and McCulloch as "oneirophrenia," is characterized

by a more or less acute onset with the symptomatology similar to the first group except for the impairment of the sensorium with confusion and disorders of perception and recollection. The symptoms are reversible, and the prognosis is generally good. This disorder is said to be due to a disturbance in carbohydrate metabolism (see below).

It is not entirely clear, but seems possible that Meduna and McCulloch's "schizophrenia" corresponds to Sullivan's "dementia præcox" and their "oneirophrenia" to Sullivan's "schizophrenia." What is to Sullivan a "disorder of living" is to Meduna and McCulloch a "disorder of carbohydrate metabolism."

HEREDITY. It is inevitable that lack of agreement regarding etiology and classification should be accompanied by divergent opinions regarding heredity. The most exhaustive recent study of heredity in schizophrenia is that of Kallmann,²⁹ whose material included 1087 schizophrenic patients admitted to the Berlin-Herzberge Hospital between 1893 and 1902 with their 3279 parents, husbands and wives, 3384 direct descendants, 3920 brothers, sisters, half-brothers and half-sisters, and 2194 nephews and nieces. One gains the impression that the author has set about his task with the intent of proving something of which he had already convinced himself. This may perhaps be gathered by a preliminary statement, "The principal aim of our investigation was to offer conclusive proof of the inheritance of schizophrenia . . ."

According to Kallmann, the probable incidence of schizophrenia among the offspring of schizophrenics is 19 times greater than in the general population. The grandchildren, nephews and nieces are about 5 times as likely to become schizophrenic as the average person. Qualitative biologic differences were found between the hebephrenic and catatonic types (the "nuclear" group) and the paranoid and simple types (the "peripheral" group), the simple type including cases with a mild, episodic course without marked

tendency to deterioration. The expectation of schizophrenia for an undifferentiated group of schizophrenic offspring of schizophrenic patients was 16.4%, for siblings 11.5%, for half-sisters and half-brothers 7.6%, for nephews and nieces 3.9%, and for grandchildren 4.3%. In the "nuclear" group the expectancy figures are 20.9% for offspring and 12.9% for siblings, the first figure being about double and the second about $1\frac{1}{2}$ times the corresponding rates in the "peripheral" group. Kallmann expresses the belief that the pathogenesis of schizophrenia represents a complex, though specific, hereditary process. He assumes that the genotype of schizophrenia is a single recessive trait, penetrating with a probable manifestation of about 70%. "The mode of manifestation seems to depend on the total constitution, which probably determines the disease form in the individual and is in turn conditioned by special hereditary factors." Kallmann's statistics led him to the conclusion that no biologic or hereditary relationship exists between schizophrenia and other abnormalities such as epilepsy, neurosyphilis, feeble-mindedness, alcoholism and criminality.

Kallmann advises the employment of extensive eugenic methods for eradication of the disorder. Sterilization of schizophrenics themselves would prevent but 1.7 to 3.4% of the total number. Eugenic prophylaxis should therefore be directed toward the tainted families; in certain cases there should be legal power to intervene. The "establishment of State archives of the tainted families" is also recommended.

Barahal,³ in a brief study, agrees with Kallmann that the genetic analysis of schizophrenia shows a recessive inheritance of the main gene concerned, "but an irregular expression of the gene due to the modifying effects of both secondary genes and various environmental influences." He believes that while mental hygiene, psychotherapeutic and shock measures should be utilized, schizophren-

ics and their closest blood relatives should be discouraged from bearing children.

Pollock, Malzberg and Fuller,⁴⁴ using data derived from the New York State psychiatric hospitals, agree that schizophrenia arises more frequently in tainted families. They state that the type of psychosis, whether schizophrenic or manic-depressive, is more related to family history than to the precipitating situation. Malzberg³⁸ believes, however, that a single recessive factor, as suggested by Kallmann, does not adequately explain the inheritance. The fact that in Kallmann's study the incidence of schizophrenia in offspring is greater than in the siblings, has also led to the denial of the recessive character of the heredity and the postulation of a dominant characteristic (Mayer-Gross and Moore³⁹).

Terry and Rennie,⁵⁰ in a comprehensive study of 77 patients, were impressed with the role of familial incidence in the background of their patients. The study lacks, however, any control material. In only 4 cases was no abnormality reported in the background. In 61 cases there was some type of familial psychiatric disorder; in 40 of these the disturbance was in the parents. The parents had actual psychoses in 23 cases, but in only 5 of these was it schizophrenic. In 29 cases there were actual psychoses in atavistic or collateral relatives. In an additional 12 cases, 1 or more relatives exhibited various character abnormalities. The authors conclude, "In considering the constitutional make-up of our patients, we are forced to accept the evidence of familial instability as well as to realize that these psychotic and unstable relatives contribute to the insecurity of the environmental background in which these patients were developing."

PSYCHOGENESIS. In Terry and Rennie's evaluation of 77 patients⁵⁰ with detailed case studies, environmental factors "emerge as extremely important in the development of personality features that make for difficulty. Equally evident is the fact that their families fell into extremes of solicitude and over-attention on

the one hand and, on the other, states of nagging, pushing and comparison with better-adjusted siblings calculated to increase the gap between this child and the others or the outside world. Difficulties between the parents or the deprivation of a parent seems to contribute further to the insecurity of these children. The parental make-up is extremely important, and we find a large number of these children with the added disadvantage of daily contact with psychotic, eccentric, or emotionally unstable parents. A prominent feature of the family constellation is the pushing and nagging to which these children were exposed in an attempt by their families to have them reach the achievements for which they were not equipped, or an attempt to keep them apace with more gifted or successful brothers and sisters. In these children we find ample evidence of childhood instabilities being further subjected to environmental stresses making for increased uncertainty, insecurity and instability. It is impossible to study this material without being impressed by the importance of the family constellation and of the effects of maladjustments within the family in contributing to the growing timidities and insecurity of the children."

Of the 77 patients, 60 showed states of dependence on family overmanagement or overattachment to the family. Of the 60, 52 exhibited such childhood difficulties as enuresis or "delicate health." Of the remaining 17 patients, all but 1 showed evidence of family difficulties such as undue emotional attachment, poor emancipation, negativistic attitudes or poor adjustment to loss of a family member. The mother played the most significant rôle in the overdependence, somewhat more frequently in the male patients than in female patients. Presumably associated with the frequency of family dependency, resentment states, and difficulties in achieving emancipation, 71 of the 77 patients never attempted or had difficulties with ventures outside the family (scholastic, occupational, marital). Per-

sonality difficulties were accentuated in marriage. Difficulties and inadequacies were evident in the sexual sphere in 58 cases. Of these patients, 34 were either aloof or deliberately avoided contact with the opposite sex. In 22 cases autoerotism was a focus of anxiety, while homosexuality played a similar rôle in 15 cases.

Fromm-Reichmann,¹⁴ choosing to ignore or minimize constitutional factors, explains the development of the schizophrenic state on the basis of early infantile traumatic experiences, in contrast to those occurring in later childhood as found in psychoneurotics. "We think of a schizophrenic as a person who has had serious traumatic experiences in early infancy at a time when his ego and its ability to examine reality were not yet developed. These early traumatic experiences seem to furnish the psychologic basis for the pathogenic influence of the frustrations of later years." Such early traumata inflict greater damage on the personality than those occurring later.

"In addition, early traumatic experience shortens the only period in life in which an individual ordinarily enjoys the most security, thus endangering the ability to store up, as it were, a reasonable supply of assurance and self-reliance for the individual's later struggle through life. Thus is such a child sensitized considerably more towards the frustrations of later life than by later traumatic experience. Hence, many experiences in later life which would mean little to a "healthy" person and not much to a psychoneurotic, mean a great deal of pain and suffering to a schizophrenic. His resistance against frustration is easily exhausted. Once he reaches his limit of endurance, he escapes the unbearable reality of his present life by attempting to reëstablish the autistic, delusional world of the infant, but this is impossible because the content of his delusions and hallucinations are naturally colored by the experiences of his whole lifetime."

NEUROPATHOLOGY. In spite of many years of observation, no consistent abnor-

malities in brain structure have been noted. The studies of early neuropathologists, especially Alzheimer, showing cortical ganglion cell degeneration and fatty deposition have been verified by some recent observers and refuted by others. The attitude toward the histologic findings seems more determined by the training and orientation of the observer, whether he is neuropathologically or psychologically inclined, rather than the actual evidence.

The confused thinking in this regard is well illustrated in Hassin's textbook of neuropathology.²³ He introduces the subject by stating, "The several clinical forms of the mental disorder known as schizophrenia or dementia præcox are considered organic diseases of the brain, and not functional disorders . . ." This is followed by a description of a variety of pathologic observations "not uniform or typical enough to enable one to make a diagnosis under the microscope," including ganglion cell changes in third, fifth and sixth layers of the cortex, occasional foci of gliosis and accumulation of lipoids. In contrast to the preliminary assumption, Hassin concludes, "It is rather significant that regardless of the enormous effort and time spent on the study of the pathology of dementia præcox, the results are insignificant. . . . It is questionable, whether even the changes described above are not secondary, due to the long duration of the disease with its unavoidable somatic complications."

A number of reports have appeared recently concerning patients with schizophrenic syndromes in which definite and severe neuropathologic features were found at postmortem examinations. One of the most interesting is that of Holt and Tedeschi.²⁵ The patient had been in a state hospital in 1924 for 5 months with the diagnosis of catatonic dementia præcox and was discharged as completely recovered. Eighteen years later he again developed a severe catatonic episode and died. There were no abnormal neurologic findings during his illness. Histologic studies

disclosed a primary demyelinating process characterized by sharp demarcation and limitation of lesions to the cerebral white substance. Polatin *et al.*⁴³ describe 2 patients, hospitalized over long periods of time for schizophrenia, who showed signs of cerebral atrophy in pneumo-encephalograms and degenerating nerve changes on biopsy study. Interestingly enough, 1 of the patients was improved following electric shock therapy.

Roizin *et al.*⁴⁶ report an acute symmetric demyelinating process in a woman who died after a brief acute psychosis resembling catatonic schizophrenia. "The relationship between the clinical symptoms and the pathologic changes is emphasized not to create an organic concept of schizophrenia but merely to illustrate that a demyelinating process acting as a somatogenic factor may precipitate, in certain cases, an acute mental syndrome, which may or may not be associated with fleeting neurologic symptoms, the evaluation of which is at times difficult." The authors postulate that certain individuals have a constitutional predisposition to react with schizophrenic pattern whether the precipitating stimulus be psychogenic or organic disease of the brain. They suggest that the 1924 catatonic episode of Holt and Tedeschi's case was precipitated by psychogenic stimuli and the later catatonic episode by the demyelinating process.

Ferraro, quoted by Roizin *et al.*, has emphasized that the following possibilities must be considered where pathologic changes are discovered in cases of schizophrenia: "(a) The changes may be the expression of organic complications in the course of schizophrenia; (b) the changes may be the expression of a primary organic disease; (c) the changes may be the expression of the composite picture, in which soma and psyche integrate each other. The acceptance of the first or the second possibility may be confusing or misleading unless the third possibility is first evaluated and accepted or discarded. The third interpretation stems out of the concept that schizophrenia must be view-

ed from the standpoint of psychosomatic integration. The organic cerebral change could therefore be considered neither as the cause nor the complication of schizophrenia, but as the result of interplay of soma and psyche in the determination of the structural pathologic process."

BIOCHEMISTRY, PHYSIOLOGY. Chemical studies of isolated blood constituents have not resulted in significant contributions to the understanding of the schizophrenic process. Recently, but few such studies have appeared. Katzenelbogen and Snyder³² have analyzed the blood serum for sodium, potassium, calcium, magnesium and inorganic phosphorus and chloride and found no significant differences as compared with a control group. The blood bromine content has been found to be lower in schizophrenic subject (0.55 mg. %) than in normals (0.81 mg. %) (Wikoff *et al.*³³), but no suggestion is made concerning the significance of the finding. Limited progress appears to have been made, however, utilizing more dynamic, physiologic methods.

Katzenelbogen *et al.*³¹ studied the dextrose content of arterial (femoral) and venous (internal jugular) blood. They found the dextrose values of both the arterial and venous blood to be lower in their group of schizophrenics than in a normal control group. However, the difference between arterial and venous dextrose values was greater in the schizophrenics than in the controls. The authors suggest that at least certain schizophrenic patients have a lower carbohydrate metabolism than normals. The higher differences in dextrose between arterial and venous blood, however, suggest a more intense carbohydrate metabolism in schizophrenics. Some of the patients had a lower oxygen content of arterial and venous blood and a lower arterial-venous difference in oxygen than in a control group, indicating a diminished intracranial oxygen metabolism. The average arterial-venous difference in carbon dioxide content was also lower. Such analyses are of limited value in

view of the small number of patients studied (12), the lack of significant deviations and statistical control, the paucity of clinical data, the conflicting results, and the fact that the control group of "normals" was collected by other workers (Gibbs *et al.*).

Horvath and Friedman²⁶ noted a delay in the hypoglycemic effect and the subsequent recovery after intravenous administration of insulin in schizophrenic patients when compared with normal subjects. Glucose tolerance curves were found by Proctor *et al.*⁴⁵ to have an abnormally prolonged interval before returning to fasting levels, indicating a decreased glucose tolerance. These curves tended to return to normal following recovery, regardless of the type of therapy employed. Patients with apparent recoveries in whom the glucose tolerance curve did not improve often relapsed and the authors feel that the test may be of some prognostic importance.

The cases with acute onset with clouding of consciousness described by Meduna and McCulloch⁴⁰ as "oneirophrenia" (see above) are said by these authors to be associated with a disordered carbohydrate metabolism. This disorder is characterized by a diabetic-like Exton-Rose glucose reaction, a slow return to normal of the blood sugar curve, resistance to insulin, and an abnormally large output of an anti-insulinic factor in the urine. (Meduna had previously shown that 60% of schizophrenic patients had an anti-insulin factor in their blood.) The carbohydrate metabolism returns to normal with the disappearance of symptoms. It is apparently assumed by these authors that the metabolic defect is the causative factor.

Gellhorn,¹⁷ on the assumption that schizophrenia is associated with deficient reactivity of the sympathetic nervous system, has attempted to show that all "successful" treatments of schizophrenia are effective because of stimulation of the sympathetico-adrenal system. This is said to be accomplished for the most part by the production of a lowered oxidative

metabolism of the brain. That a relative anoxia leads to increased sympathetic discharge is shown by the rise in systemic blood pressure that occurs upon breathing a gas mixture low in oxygen. Gellhorn's experiments show that insulin hypoglycemia diminishes cerebral oxygen consumption and thus leads to sympathetic stimulation. Metrazol, even when convulsions are eliminated by curarization, acts similarly. The combination of hypoglycemia and oxygen deficiency produces greater sympathetic stimulation than even complete asphyxia. "If, as has been shown, successful treatment of schizophrenia is in essence due to nothing other than strong and lasting excitation of the sympathetic division of the autonomic system, involving profound alteration in the oxidative metabolism of the brain, it is to be expected that deficiency in the reactivity of the sympathetic system is a basic feature of schizophrenia."

In a later study Gellhorn *et al.*¹⁸ find that normal and schizophrenic patients have the same amount of insulin and glucose in the blood in the absence of emotional excitement. During emotional excitement, however, the insulin content of the blood is greatly increased in psychotic patients and in most instances the blood sugar remains unchanged. When the blood is injected into a test animal, the increased insulin concentration leads to a marked fall in blood sugar and coma. Normal persons, on the other hand, show a slight rise in blood sugar with emotional excitement and their blood produces no hypoglycemic reaction in the test animal. "The experiments are interpreted to mean that the balance of the autonomic centers in psychotic patients under emotional stress is shifted toward the vago-insulin side whereas in normal persons the sympathetico-adrenal system greatly predominates." These findings and conclusions are not at all consistent with those of Horvath and Friedman, Proctor *et al.*, and Meduna and McCulloch and require further validation.

A number of investigators^{19,21,22} have

noted nitrogen retention in patients suffering from periodic attacks of catatonia with normal nitrogen balance in remissions. This is said to be on the basis of faulty liver function. Liver function, as measured by the hippuric acid test, was found to be impaired in catatonic patients, while undisturbed in other schizophrenic and normal subjects.

A group of workers at Worcester State Hospital have brought together a body of physiologic data in an effort to broaden the psychologic concept of schizophrenic withdrawal to include physiologic phenomena. Schizophrenic withdrawal is defined as a state in which "the vital relations between a person and his physical and social environment become less intense and less numerous." They state that physiologic hyporeactivity is found in the general metabolism, functions of the autonomic nervous system, and functions of the central nervous system. The schizophrenic patient has (1) a lowered response to administration of thyroid;⁸ (2) a reduced thermal response to dinitrophenol, a general oxidative stimulant;⁹ (3) a lowered blood pressure and pulse rate response to the intravenous administration of epinephrine hydrochloride;¹⁰ (4) diminished responses in blood pressure, pulse and respiratory volume as a result of blocking heat loss from the lungs by breathing hot, moist oxygen;^{12a} (5) depression of the amount and of the duration of nystagmus as a result of vestibular stimulation¹ and significantly less sway following rotation than normal subjects;^{12b} and (6) resistance to insulin.¹¹ In summary, Angyal, Freeman and Hoskins² note, "We are not prepared to state whether psychologic withdrawal is caused by a physiologic withdrawal (hyporeactivity) or *vice versa*. It is probable that neither is the case, but that one is dealing with withdrawal of the total personality manifested in a variety of psychologic and physiologic characteristics." Recent studies of the disorders of thinking and language in schizophrenia, while not solving the

problem of etiology, have contributed to the understanding of schizophrenic behavior.

Cameron⁷ has made an experimental analysis of the disorders of thinking and language in schizophrenia. This analysis leads him to describe as characteristic of schizophrenic disorganization: (1) Asyndetic thinking: This is marked by a lack of essential connections in the use of language. In place of well-knit sequences, the schizophrenic gives only a half-organized collection of fragments. (2) Metonymy and personal idiom: Unprecise substitute terms or phrases are used in place of exact terminology. (3) Interpenetration of themes: Parts of one theme appear as intrusive fragments in another unrelated theme. "Such a situation, in which a patient's asocial fantasy themes are able continuously to subordinate all external events in the field of social behavior, provides the patient with only a distorted and fragmented environment, which cannot possibly influence his conduct in a socially organized way." (4) Overinclusion: "These disorganized schizophrenics could not manage the essential first step in problem solving, that of narrowing down one's operations to something restricted and unified enough to call out organized attitudes and specific responses." (5) Incongruity between acts and words. (6) Altering the experimental conditions of psychologic tests: "It is suggested that this trend may be related to the patients' general inability to deal with facts as they are and to their tendency, when difficulties arise in the social field, to fall back upon some substitute in fantasy." (7) Generalizations varied but ineffectual. These generalizations concerning the solution of psychologic test problems were not useful because they were either too inclusive, too involved or too entangled with fantasy.

DETERIORATION. Dementia and deterioration have generally been employed synonymously and have usually implied a permanent impairment of intellectual function. Progressive intellectual deterior-

ration was to Kraepelin an essential feature of "dementia præcox." Though denied as inevitable by Meyer, Bleuler and other psychiatrists during the early portion of the century, the concept of deterioration has continued to be commonly accepted as a definite feature of at least certain cases. Numerous psychologic studies during the past 30 years have shown that the disturbance in thinking observed in the schizophrenic process is not identical with that seen with organic brain disease or feeble-mindedness and that the disturbance is not necessarily an irreversible one. Among some of the more recent discussions of deterioration in schizophrenia are those of Kendig and Richmond³³ and Cameron.⁷

The study of Kendig and Richmond is based on an analysis of the psychologic examinations given at St. Elizabeth's Hospital during a 15 year period. The median mental age of the schizophrenic group was 11 years 5 months, about 2 years below the norm for the general population. They noted that the patients as a group showed inferior learning ability even during their school years. This difficulty could not be accounted for on the basis of early onset of the psychosis. "Bringing together these specific findings, our general conclusion is that the dementia præcox mind is blunted and dulled. This intellectual inferiority is not primarily due either to deterioration or to temporary impairment resulting from the psychosis, since it shows itself in extensive school failure long before the actual breakdown and, in most cases, before the occurrence of the first premonitory symptoms of the disease. While in some instances it may be congenital, our case histories suggest that more often it is the product of the emotional maladjustments which later play an important etiologic rôle in the precipitation of the psychosis." Whether or not the basis for school difficulties is of congenital origin or due to emotional conflicts, the problem remains the same. "A personality inadequate to meet the everyday exigencies of life may, as the

stress becomes greater with the years, tend to escape, or be precipitated, into psychosis. This amply explains our test findings without implying any reduction in intelligence resulting from the disease, and thus saves us from the necessity of hypothecating somatic changes." Re-examination of 41 schizophrenic patients with the Stanford-Binet test after prolonged hospitalization indicated no progressive decay of function. In most of the patients a gradual recovery in the intellectual sphere corresponding with improvement of psychotic symptoms was noted.

Cameron⁷ has shown that the disorganized thinking of schizophrenics does not follow the pattern of either deteriorated senile patients or of normal children. Lehrman,³⁵ who concluded that schizophrenic "deterioration" was neither inevitable or irreversible, suggests that the term be reserved for those patients suffering, not from schizophrenia, but from organic brain disease.

CONCEPTUAL THINKING and "regression" hypotheses. Attempts have been made to relate the schizophrenic thinking disorder to some basic concept. One of these is the concept of "regression" to either the level of primitive man or to the level of early childhood. One of the best known descriptions of regression to primitive levels in schizophrenia is found in Storch's "The Primitive Archaic Forms of Inner Experiences and Thought in Schizophrenia."⁴⁸ Storch found great similarity between the experiences of schizophrenic patients and those of various primitive people. He conceived of modern man as having a "rational superstructure" which, as the result of a "conflict of instincts of constitutional nature" (such as perverted sexual attitudes), crumbles; and primitive magic-archaic modes of behavior and thinking well up from the substrata. Others more recently have stressed such regressive characteristics as typical of schizophrenia, including Osborne⁴² who suggested that the term schizophrenia be changed to

"palaeophrenia." Others have suggested regression to a childhood level. Gardner,¹⁶ for instance, has equated psychotic symptoms with childhood behavior and measures the regression in terms of "psychotic age."

Goldstein²⁰ has distinguished between abstract and concrete behavior. The concrete attitude is realistic and bound to the immediate experience of the given thing or situation. The abstract attitude, however, passes beyond the given specific thing or situation which then becomes merely a representative of a category. Hence the latter type of thinking is also called the categorical or conceptual attitude. Goldstein, Piaget, Vigotsky and others have concluded that childhood thinking is concrete and that the abstract attitude develops ordinarily after puberty. Goldstein has also shown that loss of abstraction occurs with organic brain disease (as discussed in a previous review³⁷).

Vigotsky⁵² studied concrete and conceptual thinking in schizophrenics by means of a block test devised by Ach. Blocks of different colors, sizes and shapes were used which the subject was to sort into 4 different categories. This sorting required the subject to form and test a number of theories or concepts. Vigotsky found that schizophrenics lacked the capacity to carry through the procedure successfully. He therefore postulated the loss of conceptual thinking as the basic psychologic disturbance in schizophrenia. Reminiscent of Storch, he conceived of the mind as being composed of an "old" substructure of concrete thinking, and of a "newer" outer layer of concepts. The latter portion of the structure is involved in schizophrenia. To Vigotsky, childhood, organic brain disease, and schizophrenia have in common the lack of ability in forming concepts. He therefore concluded that schizophrenia is an organic brain disease.

In this country, Hanfmann and Kanasin²⁴ have continued and amplified Vigotsky's work with the Ach test. They agree that conceptual thinking is impair-

ed in certain schizophrenics but believe that a transition from the highest conceptual level to the lowest primitive level of thinking is found, thus denying the simple dichotomy between the two as expressed by Vigotsky. The highest conceptual level of thinking demonstrated by the block test is found only in Hanfmann and Kanasin's college educated group, while the non-college group scored only at an "intermediate level." When schizophrenic patients were compared with normal subjects with comparable educational background, defects in concept formation were noted, but only in a portion of the schizophrenic group. Definite impairment was found in 26 of 62 subjects. It was found most commonly in patients classified by Kanasin as "primary thought disorders" (see above), and in the groups marked by dissociation with fantastic elaboration of ideas and in those with paranoid-hebephrenic trends with dull affectivity. Schizophrenic patients with neurotic traits tended to have little or no impairment in conceptual thinking.

Goldstein²⁰ has recently applied the methods he had previously used in studying patients with organic brain defects to schizophrenic patients. He agrees that concrete thinking as opposed to conceptual or categorical thinking is found in a certain number of schizophrenics. Goldstein points out that regardless of the differences in normal individuals in abstract thinking, they can learn by demonstration to proceed along an abstract way. The schizophrenic, however, cannot learn to do so. "Many of the peculiarities of the behavior of schizophrenics become understandable, when considered as expressions of a greater concreteness. Concrete behavior means that in our behavior and activity we are governed, to an abnormal extent, by the present outer world stimuli and by the images, ideas, and thoughts which act upon us at the moment. On the one hand, symptoms arising from this attitude are autistic thinking and acting, and on the other hand, abnormal boundness to outer world stimuli as far

as they belong to that realm of reality which the patient experiences. The world of the schizophrenic is determined to a pathologic extent by his own feelings and thinking, and by his capacity to react. The demarcation between the outer world and his ego is more or less suspended or modified in comparison with the normal. Here is one origin of illusions. The objects which impress the patients are not the same as those which would impress the normal person in the given situation. He experiences only objects to which he can react in the only way in which he is capable, *i. e.*, in the concrete way."

Hanfmann and Kasanin and Goldstein point out that the loss of abstract thinking found in both schizophrenic and organic brain disease is not necessarily proof of an organic origin of the schizophrenic process. Goldstein notes certain differences between the 2 groups. In the organic group, disintegration toward the concrete is of a simplified and inane form but more or less obvious to the observer. The concreteness of the schizophrenic is different, inasmuch as his thinking is richer and animated with more personalized ideas. While the similarities between the 2 groups suggests that a somatic factor is involved, the defect could also be considered as a retreat from an unbearable conflict.

Benjamin (see Kasanin³⁰) has studied the thinking in schizophrenic subjects by having them interpret a series of proverbs:

1. When the cat's away, the mice will play.
2. Don't cry over spilt milk.
3. It never rains but it pours.
4. The burnt child dreads the fire.
5. Don't cross your bridges till you come to them.
6. A rolling stone gathers no moss.
7. Discretion is the better part of valor.
8. To fiddle while Rome burns.
9. Don't count your chickens until they're hatched.
10. The proof of the pudding is in the eating.
11. He who laughs last, laughs best.
12. New brooms sweep clean.
13. Ingratitude, thy name is woman.
14. He travels swiftest who travels alone".

Completely literal (concrete) responses, such as "It doesn't stay long enough in

one place" as the response to the sixth proverb, are found much more commonly in intelligent schizophrenics than in mildly defective non-schizophrenics. Such responses are found only in schizophrenia, organic brain disease and mental deficiency. False symbolizations are also commonly noted in schizophrenics, as "a person who brags all the time never gets anywhere" in answer to proverb No. 6.

Kendig and Richmond,³³ and Cameron do not believe, however, that the loss of conceptual thinking is the basic characteristic of the schizophrenic state. The former has not found defects limited to this sphere, but rather present in all tests requiring sustained effort and attention. Cameron has found that even severely disorganized schizophrenics are capable of abstract attitudes when adequate coöperation is obtained. He is critical of the rigid interpretations required by Hanfmann and Kasanin in the Ach block test; he believes that while the patients' interpretations may not agree with those of the examiner, they nevertheless may still be generalizations. "There is good reason for doubting the usefulness, to say nothing of the validity, of these determined efforts to maintain separate categories of 'abstract' and 'concrete' behavior. The notion is based upon an equally hypothetical differentiation between 'perceptual' and 'conceptual' thinking; and upon inspection this will be found to reduce to little more than the ancient narcissistic flattery that granted rationality to adult human thought but denied it to children and animals—some stoutly denied it to women also." "The fact that schizophrenic disorders do appear among adults in 'primitive' (*i. e.*, non-technologic) civilizations and among young children of our own contemporary technologic civilizations, as well as the results of comparisons that have been made between the logic of normal children and adults and that of schizophrenics, contradicts the regression hypotheses. Moreover, the contention is supported by the same evidence that schizophrenic disorganization represents

neither a retracing of the ontogenetic-phylogenetic path of development, nor a removal of outer layers of thought to expose any 'primitive nucleus.' Instead, it presents us with a development, new and unique for a given individual's life history, that can be made quite intelligible in behavioral terms."

The schizophrenic, according to Cameron, is essentially "disarticulated" from the social group. The normal person gradually acquires language behavior and thinking which can be shared and compared with others and by which he learns to be self-critical. He feels that disorganized schizophrenics are persons who have not developed adequate role-taking skills, who have a history of social inadequacies, and who have been unable to establish themselves firmly in their cultural patterns. Faced with emotional conflicts and disappointments, they progressively withdraw from social participation into fantasy.

Sullivan (see Kasanin³⁰) has emphasized that the language of the schizophrenic is not meaningless, but has meaning only to those with an intimate knowledge of him. The language of the schizophrenic is not adequately censored by the patient or, as Sullivan puts it, lacks "consensual validation." This language defect is reminiscent of the language of dreams or the speech of very young children. The schizophrenic employs speech in a magic, autistic way in order to counteract his feeling of insecurity.

The Rorschach test, in which a series of standardized ink blots are interpreted by the patient, has been useful as a diagnostic adjunct and has contributed additional information concerning the personality structure of the schizophrenic. Because of the technical nature of the test, the subject cannot be adequately reviewed here. Some of Beck's conclusions,⁴ however, may be cited. He takes to task 2 long accepted generalizations, first that schizophrenics have a rich life in fantasy and secondly that the schizophrenics have shallow emotions. From Rorschach test performances Beck shows

that what has been conceived as fantasy is really distortion, misconstruction and inaccuracy, but not fantasy in the sense of a new creation. The emotions of the schizophrenics are not shallow but often exaggerated and poorly controlled.

THERAPY. In contrast to the numerous and diverse publications dealing with "organic" therapies for schizophrenia, the field of psychotherapy is marked by a paucity of recent contributions. This situation does not adequately reflect the actual status of psychiatric practice in many of the outstanding psychiatric institutions in this country. The writing of the numerous articles concerned with "organic" therapies has been promoted by the ease with which large groups of patients are collected, the appeal of new and rather easily learned treatments, and the rapid and dramatic response of many patients. While the various shock therapies have been generally accepted as worthwhile therapeutic adjuncts, they have not as yet contributed a great deal to our understanding of schizophrenia. Sullivan,⁴⁹ looking upon both psychoneurosis and schizophrenia as the outcome of difficulties in interpersonal relationships, stresses a similar therapeutic approach in both modified to the needs of the individual patient. He objects strenuously to the various "organic" treatments on the grounds that they reduce "the patient's capacity for being human," a fact that has not been verified for the shock therapies. Fromm-Reichmann,¹⁴ whose thesis of the origin of schizophrenia as the result of psychologic traumatic experiences in early childhood has already been noted, also stresses that analytical procedures may be profitably employed. The patient must feel comfortable and secure enough to give up his narcissistic isolation and to use the physician for resuming contact with the world. "It is certainly not an intellectual comprehension of the schizophrenic but the sympathetic understanding and skillful handling of the patient's and physician's mutual relationship that are the decisive therapeutic factors."

The 2 most frequently employed shock therapies, insulin coma and electrically induced convulsions, have been previously described in this section^{27,53} and elsewhere and will not be discussed in detail here. The report of Bond and Rivers⁶ is representative of the results with insulin shock therapy. In a control group of 100 patients not treated with insulin shock, 16 were recovered or much improved after hospitalization; 14 of these were well at the end of 6 or 7 years. Of 251 schizophrenic patients treated with insulin shock, 138 (54%) were recovered or much improved at the end of treatment; but only 41% maintained their status over a 5 year period.

Reports of results with electric shock treatment of schizophrenics have varied from 10% improvement (Smith *et al.*⁴⁷) to 70% improvement (Kalinowsky²⁸). Kalinowsky emphasized the importance of continued treatment in spite of early clinical improvement, frequently employing 20 or more shocks in contrast to the 10 or so used by others. "Discontinuance of treatment after the usually early clinical improvement leads almost invariably to relapse and is the most important reason for failure of the method in the treatment of schizophrenia."

Other procedures which have recently been suggested in the treatment of schizophrenia include bilateral prefrontal lobotomy ("psychosurgery"¹³), "electronarcosis"⁵¹ and "pyroelectric shock therapy."¹⁵

PROGNOSIS. All agree that acute onset and short duration of the psychosis are favorable prognostic signs. Terry and Rennie⁵⁰ have summed up the factors contributing to a good prognosis in schizophrenic patients as: "Sympathetic environment to which they can return; interests, goals, and pursuits in keeping with their ability; onset of the psychosis in a setting of unusual strain possible to modify; intact rapport and the combined will and ability to cooperate. Special features of the psychosis that seem prognostically valuable are evidences of intact thinking and personal appearance, absence of hallucinatory

phenomena, affective admixtures in the psychoses, or the presence of a temporary infectious process at the onset or during the psychosis."

Lewis³⁶ has studied prognosis from the standpoint of prepsychotic factors (heredity, morphologic type, character, social factors, intellect and trauma), the initial stage of the psychosis (age at onset, insidious or sudden onset and exogenous precipitating factors), the symptoms of the acute stage, and the course of the disorder (steadily progressive, tendency to remission, catastrophic development). Some favorable prognostic factors are said to be: pyknic habitus, cycloid temperament, vigorous emotional response (especially depression) at the onset, active exogenous precipitating factors, and amnesia for the acute phase.

Summary and Conclusions. The term schizophrenia should be used to designate, not an isolated disease entity, but a psychiatric reaction seen in certain ("susceptible") individuals as the result of a more or less prolonged difficulty in adjustment—a "disorder of living." An all-inclusive definition of the term is difficult because of the myriad patterns of expression in different individuals and, often enough, in the same individual at different times. Common findings in the schizophrenic reaction include vague, autistic thinking, lack of interest and progressive withdrawal from the realities of life, peculiarities of emotional expression or an *apparent* emotional indifference, delusions and hallucinations, *apparently* incoherent or irrelevant language, and bizarre motor behavior such as catatonia.

The attempt to pigeonhole schizophrenic patients into a number of specific categories has been relatively unproductive. No present classification contains mutually exclusive subdivisions. We are not even certain of any clean-cut demarcation between the psychoneurotic reaction and schizophrenia on the one hand and between the manic-depressive reaction and schizophrenia on the other. More understanding is to be gained by studying the

patient and his life as an individual problem rather than by studying him as a representative of a nebulous subdivision.

The suggestion has been made that 2 different types of disorder are included in the total group. One is said to be an insidious, chronic malady with a uniformly poor prognosis and is presumed to be due to deteriorative disease of the central nervous system. The other type is said to have a more acute onset, a relatively good prognosis and to be of psychogenic origin. There is little question that the prognosis is poorer when the onset is insidious and when the disorder has been present for a long period of time; this applies to either treated or untreated patients. Hence, such a thesis may be of some practical importance in the treatment of schizophrenic patients. There is, however, no adequate proof that 2 etiologically different groups exist or that the difference between the 2 is a qualitative rather than a quantitative one.

The search for a single etiologic factor has been uniformly disappointing to about all but the individual proponent of his own theory. The inheritance of schizophrenia has been said to follow one or the other type of Mendelian transmission. Suggestions have been offered that the inheritance is of the simple recessive type, the dominant type, or of a mixed type in which a number of primary and secondary genes play a rôle. While there is little doubt that psychiatric disturbances are more frequent in the family of schizophrenic patients than in "normals," the number of divergent views of those favoring an hereditary origin is in itself evidence that present statistical evidence is inconclusive. We are unable to state with any degree of certainty the relative rôles of inheritance (or "constitution") and the effect of psychiatrically handicapped relatives as environmental liabilities contributing to maladaptation. It is probable, however, that the relative rôle of each varies with each patient.

The results of both neuropathologic and metabolic studies are so conflicting that

the unbiased observer is all but overwhelmed by confusion. The few variations that have been noted with any degree of consistency may prove to be as readily explainable as the effect rather than as the cause of the disorder. It is well to bear in mind, however, that a number of organic diseases involving the cerebrum may precipitate a schizophrenic syndrome, and differential diagnosis may be difficult. This is especially true of catatonic syndromes.

Studies of the life history of schizophrenic patients have without doubt thrown more light upon the understanding and treatment of the schizophrenic reaction than all of the multitudinous hereditary, pathologic and metabolic researches. Such studies are time-consuming and difficult to express statistically. Yet but few who take the time to carry them out can help but be convinced of the importance of life factors in contributing to the insecurity and maladjustment of the individual and the development of the schizophrenic reaction.

Such factors, however, have not offered the entire solution to the problem of etiology. We have inadequate psychologic explanations to explain the development of psychoneurotic reactions in certain individuals and of schizophrenic reactions in others as the result of apparently similar psychogenic stresses; indeed, we have no proof that these same psychogenic stresses may cause no particular psychiatric disturbance in still others. The postulate that the psychologically traumatic experiences of schizophrenics appear earlier in life than those of psychoneurotics can neither be affirmed nor denied with certainty. We are, at least for the present, forced to lean upon the somewhat vague concept of "constitution," by which is generally meant the inborn, hereditary attributes that influence personality structure. Present-day studies correlating morphology and personality may help to clarify this concept.

A great deal of effort has been expended in studying the language, thought and

behavior of schizophrenic patients. It is clear that the language of even the most disorganized schizophrenic is not meaningless. An intimate knowledge of the person and his life may be necessary, however, before the meaning becomes apparent to the observer. Numerous attempts have been made to relate schizophrenic thinking to that of children or of primitive peoples. Hence, in some quarters, schizophrenia is considered a "regressive" disorder. Recently this viewpoint has been rekindled by evidence that schizophrenic language is similar to that of the child or of primitive man in that it is "concrete." There is said to be a defect in "conceptual" (or abstract or categorical) thinking. While this defect is unquestionably present in many instances, considerable doubt remains as to whether it is a basic factor. The fact that a proponent of this viewpoint finds definite impairment in conceptual thinking in less than one-half of a group of schizophrenic patients indicates that it can hardly form the basis for an all-inclusive hypothesis. There is no convincing evidence that patients with impairment of conceptual thinking have a fundamentally different disorder than those in whom it is intact.

Psychometric tests indicate that intellectual disturbances in schizophrenia are not similar to those with organic brain disease. Any apparent reduction in intelligence may be adequately explained in

most instances on the basis of emotional maladjustments. Long term psychological studies of schizophrenic patients show that this apparent intellectual impairment is capable of recovery even after long periods of time. The terms "deterioration" or "dementia," which have been extensively employed, seem to have no useful place in the concept of schizophrenia and had best be reserved for patients suffering from organic brain disease with intellectual dilapidation.

Both shock therapies (insulin coma and electrically induced convulsions) and psychotherapeutic methods have their place in the treatment program. There is, unfortunately, the tendency to employ shock therapy without reference to the personality problems of the patient. The use of isolated shock therapy has undoubtedly contributed to the large number of relapses. On the other hand, reliance solely upon present methods of psychotherapy may necessitate a prolonged and expensive hospitalization which can only apply to the select few. Unfortunately, at the present time no adequate studies of shock therapy combined or followed with intensive psychotherapy have appeared. If the concept of schizophrenia as a "disorder of living" is correct, the isolated use of any shock procedure without reference to the personal and interpersonal problems of the individual patient cannot be expected to lead to long-term improvement.

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PHYSIOLOGY

PROCEEDINGS OF

THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA

SESSION OF MAY 21, 1946

Effect of Concussion Upon the Polarizability of the Brain. E. A. SPIEGEL, M.D., G. C. HENNY, M.D., H. T. WYCIS, M.D., and M. SPIEGEL-ADOLF, M.D. (Depts. of Experimental Neurology, Colloid Chemistry and Physics, Temple Univ. School of Medicine, Philadelphia, Pa.). The polarizability of the cerebrum was studied in cats and guinea pigs following concussive and subconcussive blows produced by a pendulum. After subconcussive blows, no significant change of polarizability was found, while a significant decrease occurred following severe concussive blows. This change indicates an injury to cell membranes in the cerebrum; it is a reversible process, and it appears also in animals kept under artificial respiration. The impairment of the polarizability of the cell membranes, together with some changes affecting the interior of the cells, belongs to a group of secondary changes reaching a maximum after the fleeting functional disturbances characteristic for concussion have subsided. (Appeared in *Am. J. Physiol.*, 146, 12, 1946.)

Direct Measurement of Coronary Blood Flow in the Anesthetized Dog. J. E. ECKENHOFF, M.D., and C. M. LANDMESER, M.D. (Dept. of Pharmacology and Harrison Dept. of Surg. Research, Univ. of Penna.). In heparinized dogs anesthetized with nembutal or morphine-chloralose, blood was circulated from a carotid artery through a bubble flowmeter into a cannulated left descending or circumflex coronary artery. The chest was then closed and spontaneous respiration resumed. Flow readings before and after closure of the chest showed significant differences. Under the conditions of these

experiments the mean "normal" coronary blood flow was 64 cc. per 100 gm. of heart tissue (coefficient of variation ± 11.2). A striking parallelism of blood pressure and coronary blood flow was observed, a given percentile drop in blood pressure usually resulting in an approximately equal percentile drop in flow. Electrical stimulation of cardiac sympathetic and parasympathetic nerves gave no evidence of vasoconstrictor innervation to the coronary vessels. Epinephrin injected into the coronary artery produced immediate and brief increases of flow independent of change in blood pressure and pulse rate. Acetylcholine intra-arterially had similar effects tending to be more marked and more transient. When the common vasodilators were given systemically, papaverine intravenously produced the most significant and sustained increase in coronary flow with a minimum of side reactions. Aminophylline intravenously acts much the same but caused a prolonged increase in pulse rate. Amyl nitrite inhalation produced marked increases in flow (even with decreases in blood pressure) and violent increases in pulse rate. Nitroglycerin, intravenously, intramuscularly and sublingually was less dramatic and its effects were more dependent upon the maintenance of blood pressure. If the pressure fell, the coronary flow decreased. Nikethamide was found to be without significant coronary vasodilator effect when administered intravenously in dosage ranging from 125 to 750 mg. Preliminary observations upon cardiac metabolism indicate the average arteriovenous (coronary sinus) oxygen difference to be 12 to 15 vol. %. The simultaneous systemic (right auricular or ventricular) arteriovenous difference was

about 7 vol. %. There is no significant alteration of the coronary A-V difference under nembutal, cyclopropane, or morphine anesthesia. The oxygen uptake for the normal "resting" heart appears to be about 6 cc. per 100 gm. of heart per minute.

Cerebral Blood Flow and Cerebral Oxygen Consumption in 5 Patients With Hypertension. SEYMOUR S. KETY, M.D., and CARL F. SCHMIDT, M.D. (Dept. of Pharmacology, Univ. of Penna., and Medical Services, Phila. Gen. Hosp.). A knowledge of cerebral hemodynamics should be of considerable importance in the study of arterial hypertension for a number of reasons. Cerebrovascular complications are of great frequency in the disease and many of the symptoms are directly referable to the brain. Recent studies of renal physiology in hypertension have served to diminish the importance of the kidney as a primary etiologic factor in this disease. At the same time the experiments of Fishback and associates, and of Novak and Walker, who produced intense and persistent hypertension by artificial restriction of cerebral blood flow have reawakened interest in the rôle of central mechanisms in this disease. There have to date been no definitive studies on cerebral blood flow and metabolism in clinical hypertension; the only attempts which have been made in this direction by Raab (*Ztschr. f. klin. Med.*, 115, 577, 1931) and Williams and Lennox (*Quart. J. Med.*, 8, 185, 1939) used cerebral arteriovenous oxygen differences as measures of cerebral blood flow, a presumption which is unwarranted without concomitant knowledge of cerebral oxygen consumption. In the present study quantitative measurements of cerebral blood flow were obtained in 5 patients by the use of the nitrous oxide method previously described by the authors (*Am. J. Physiol.*, 143, 53, 1945). Mean arterial blood pressure was determined by a damped mercury manometer attached to a needle in the femoral

artery. Cerebral oxygen consumption was calculated from the values for cerebral blood flow and the cerebral arteriovenous oxygen difference and cerebral vascular resistance from the cerebral blood flow and the mean arterial blood pressure. In the 5 patients thus far studied the following mean values were found: auscultatory blood pressure 190/122 mm. Hg, mean arterial blood pressure 143 mm. Hg, cerebral blood flow 64 cc. per 100 gm. of brain per minute, cerebral O₂ consumption 3.6 cc. per 100 gm. per minute and cerebral vascular resistance 2.3 mm. Hg per cc. blood flow per 100 gm. per minute. These values may be compared with the means of 35 experiments on 15 normal young white males where values were found of: 110/80 mm. Hg, 92 mm. Hg, 70 cc. per 100 gm. per minute, 4.3 cc. per 100 gm. per minute, and 1.2 mm. Hg per cc. per 100 gm. per minute respectively. With the limitation in mind that the hypertension patients were somewhat older than the control group and included 4 Negroes, it is seen that there is a slight reduction in the average cerebral blood flow and cerebral oxygen consumption and a marked increase in the cerebral vascular resistance of the hypertensives as compared to normal. Whether any of these changes are statistically significant must await the results of a larger series of hypertensives and a more comparable group of normals. In the small series reported it is interesting to note that the cerebral blood flow decreased and cerebral vascular resistance increased with increasing severity of retinoscopic changes, lending credence to the clinical impression that retinal vascular changes reflect those occurring in the brain. The progressive but only moderate decrease in cerebral blood flow as the disease progresses in this small series is exactly analogous to the findings of Goldring *et al.* (*J. Clin. Invest.*, 20, 637, 1941) in renal blood flow. The marked increase in cerebral vascular resistance which we have found and its similarity to the changes in renal vascular resistance contributes additional evidence to the con-

cept that the generalized increase in vascular resistance occurring in hypertension is not the result of sympathetic vasoconstriction since extensive work on animals indicates a relatively weak sympathetic constrictor innervation to cerebral vessels (*Physiol. Rev.*, 16, 545, 1936).

Phase-Boundary Potentials and the Nerve Impulse.* T. CUNLIFFE BARNES, D.Sc., and R. BEUTNER, M.D. (Depts. of Physiology and Pharmacology, Hahnemann Med. Coll. and Hosp. of Phila.). 0.05 % acetylcholine produces typical spike potentials on a cholesterol-resin-guaiacol layer (less than 0.01 mm. thick) in saline recorded by an electroencephalograph through Ag-AgCl electrodes in saline on each side of the membrane. The following measurements on thick (2 cm.) oil layers show that acetylcholine is the only substance in cholinergic nerve capable of producing the action current. 0.05 % acetylcholine on cholesterol guaiacol gives 35 mv. negative (for great dilutions the sensitive benzoate-nitrobenzene interface must be used). 1 % KCl on nitrobenzene gives only 10 mv. negative, 4 % sodium lactate on cholesterol guaiacol gives 9 mv. positive, 0.1 % dibasic sodium phosphate on guaiacol gives 10 mv. positive, 0.05 % choline on cholesterol gives 15 mv. negative. 0.1 % sodium acetate gives only 3 mv. positive on guaiacol. Small afterpotentials in nerve may result from these products.

0.02 % prostigmine (the most electrically active drug tested) generates 48 mv. negative on cholesterol guaiacol: 0.05 % eserine gives 27 mv. negative. Measurements by I. Mauer show that 0.05 % dilantin and also phenobarbital sodium give 20 mv. positive which reduces the acetylcholine negativity two-thirds explaining their action in grand mal. Tridione, bromide, bromural and metrazol give no phase boundary potentials.

After the passage of an imposed current

of 6 volts (less than 1 ma.) the acetylcholine potential on oil is reduced one-third. Electroshock therapy probably alters acetylcholine brain potentials (our EEG's taken on patients after electroshock show slow waves).

One in 10,000 acetylcholine produces 25 mv. negative on frog sciatic and brain and lobster nerve in isotonic glucose (to eliminate short-circuits by salts).

Nachmansohn (*J. Neurophysiol.*, 9, 9, 1946) poisoned squid nerve with eserine, strychnine, procaine and cocaine and found no evidence for the esterase theory. If esterase is essential for the action current, eserine should prolong only the descending part of the spike but only a general toxic effect was obtained. The esterase theory also fails to explain adrenergic nerves which are represented in our model by triglyceride oils which give potential with sympathomimetic drugs but not with acetylcholine.

Regression of Mouse Sarcoma 37 Produced by Adrenal Cortex Extracts. LYLE V. BECK, Ph.D., and IRENE C. DILLER, Ph.D. (Dept. of Physiology, Hahnemann Med. Coll. and Dept. of Cancer Chemotherapy, Lankenau Research Inst.). Intraperitoneal injection of 0.5 cc. of Upjohn Co. beef adrenal gland extract resulted in hemorrhage in mouse sarcoma 37 within a few hours, and definite degenerative changes in the tumor cells, notably pyknosis of the nuclei. Over a period of several days there was a gradual decrease in size of the tumor. Tumors in the Carworth Farms female mice usually showed complete regression, the degenerated remains being sloughed off through the skin. Such females kept for several months failed to show reappearance of a tumor. On the other hand the regressions obtained in Carworth Farms males were usually temporary, and the percentage of complete regression in treated males was not perceptibly greater than that occur-

* This work was carried out under a grant from the American Philosophical Society.

ring spontaneously. Repeated injections of extract into males did not result in increase in percentage of complete regression. This tumor grows more slowly and the percentage of spontaneous regressions is higher in females than in males.

Similar effects of adrenal cortex principles on a rat transplantable leukemia have been reported by Murphy and Sturm (*Science*, 98, 568, 1943, and 99, 303, 1944) and on a mouse transplantable lymphosarcoma by Heilman and Kendall (*Endocrinology*, 34, 416, 1944). It is conceiv-

able that antibody reactions potentiated by adrenal cortex steroids might be concerned in the above antitumor effects, and that similar effects would not occur using primary neoplasms. Another mechanism which may be concerned in the inhibition of these particular malignant growths is the well-known shift toward protein catabolism produced by certain adrenal cortex steroids. The shift toward protein anabolism produced by testosterone might then be concerned in the ineffectiveness of adrenal cortex steroids in male mice.

BOOK REVIEWS AND NOTICES

BACILLARY DYSENTERY, COLITIS AND ENTERITIS. By JOSEPH FELSEN. Pp. 618; 145 illus. Philadelphia: Saunders, 1945. Price, \$6.00.

THIS volume presents a comprehensive survey of the acute infectious diarrheas, as well as a discussion of chronic enteric disorders which the author feels are etiologically related to them.

The first section of the book discusses the acute diarrheas, with the emphasis almost solely on *Shigella* infections. Following an opening on historical background, the epidemiologic, clinical, bacteriologic, pathologic and therapeutic aspects of the disease are presented. Much stress is laid on clinical features of these infections, and the author attempts to classify into 7 types what he regards as atypical forms of the disease. Complications are extensively discussed and analyzed. The data dealing with the case incidence of acute diarrheas are thoroughly presented. Also thoroughly discussed are the data revealing the predominance of *Shigella* infection when cases of "gastro-enteritis," etc., are analyzed etiologically. It might have been appropriate in his discussion of the acute colon infections for the author to examine the rôle of *Salmonella* organisms more thoroughly, as other observers have noted that these infections are often clinically indistinguishable from those caused by *Shigella*.

In the sections dealing with the chronic forms of colitis and ileitis, the author first de-

scribes their clinical and pathologic changes. Then he presents some detailed case studies which trace the clinical course of individuals with acute *Shigella* infection who subsequently developed chronic dysentery. These chronically ill patients the author thinks after thorough study are typical of those usually designated as chronic ulcerative colitis and chronic distal ileitis. Various other concepts concerning the etiology of chronic ulcerative colitis are briefly mentioned. The volume is profusely illustrated with charts and photographs of Roentgen rays and pathologic specimens, especially the section dealing with the chronic diseases.

It is somewhat unfortunate that the text tends to present the author's opinions one-sidedly. Especially so, since a number of hypotheses are presented with no documented basis in fact (such as the view that as many as 6 types of toxin are produced by the *Shigella* organisms; and the view that the chronic ulceration in chronic ulcerative colitis is due in most instances to secondary non-specific invaders, "chiefly the enterococcus, hemolytic and non-hemolytic *E. coli*," organisms which are part of the normal bowel flora anyway). However, if the volume is a bit less than scholarly in places, its publication at this time, with its enormous bibliography, by an investigator with such wide clinical experience in these disorders, certainly fills a great need in current medical literature, and should stimulate further study.

S. H.

NEW BOOKS

Cornell Conferences on Therapy. By HARRY GOLD, M.D. (Managing Editor), DAVID P. BARR, M.D., EUGENE F. DuBOIS, M.D., McKEEN CATTELL, M.D., CHARLES H. WHEELER, M.D. Pp. 322, 1st vol. New York: Macmillan, 1946. Price, \$3.25.

American Foundations for Social Welfare. By SHELBY M. HARRISON, General Director, Russell Sage Foundation; and F. EMERSON ANDREWS, Director of Publications, Russell Sage Foundation. Pp. 249; director of 505 Foundations. New York: Russell Sage Foundation, 1946. Price, \$2.00.

The Principles and Practice of Tropical Medicine. By L. EVERARD NAPIER, Companion of the Order of the Indian Empire; Fellow of the Royal College of Physicians of London; formerly Director and Professor of Tropical Medicine; Consultant to the Secretary of War; Visiting Lecturer on Tropical Medicine, Army Medical School; Visiting Lecturer on Tropical Medicine, Harvard Medical School, Boston; formerly Visiting Professor of Tropical Medicine, Tulane University, New Orleans; formerly Visiting Professor of Medicine, New York University. Pp. 917; 185 illus. New York: Macmillan, 1946. Price, \$11.00.

Journal of Gerontology (Non-technical Supplement), Winter No., Part I and Part II. Pp.: Part I, 152; Part II, 184; Vol. I, No. 1, published quarterly for The Gerontological Society, Inc. Springfield, Ill.: Thomas, 1946. Price, \$6.00 a year.

"To Add Life to Years, Not Just Years to Life. The authorized and official journal of the Gerontological Society, Inc., publishing each quarter, basic materials on problems of aging from the natural and social sciences and the humanities. The non-technical supplement is published in corresponding issues with digests from scientific journal, rewritten for the layman in a non-technical, easy-to-understand manner."

Doctors East, Doctors West. By EDWARD H. HUME, M.D. Pp. 278; 21 illus. New York: Norton, 1946. Price, \$3.00.

Renal Diseases. By E. T. BELL, M.D., Professor of Pathology in the University of Minnesota, Minneapolis, Minn. Pp. 434, 115 illus. Philadelphia, Lea & Febiger, 1946. Price, \$7.00.

The Biology of Schizophrenia. By R. G. HOSKINS, Ph.D., M.D., Director of Research, Memorial Foundation for Neuro-Endocrine Research, Harvard Medical School and Worcester State Hospital. Pp. 191. New York: Norton, 1946. Price, \$2.75.

German for the Scientist. By PETER F. WIENER, Modern Language Master at Rugby School (formerly Tutor in German in the University of London). Pp. 239. American ed. Brooklyn, N. Y.: Chemical Publ. Co., 1946. Price, \$3.50.

A History of Medicine. By DOUGLAS GUTHRIE, M.D., F.R.C.S. (Edin.), F.R.S.E. Pp. 449; 72 illus. Philadelphia: Lippincott, 1946. Price, \$6.00.

A Textbook of Biochemistry. By PHILIP H. MITCHELL, Ph.D., Robert P. Brown Professor of Biology, Brown University. Pp. 640; 69 illus. New York: McGraw-Hill, 1946. Price, \$5.00.

NEW EDITIONS

Electrocardiography. By LOUIS N. KATZ, A.B., M.A., M.D., F.A.C.P., Director of Cardiovascular Research, Michael Reese Hospital, Chicago, Ill.; Professorial Lecturer in Physiology, University of Chicago, Chicago, Ill. 2nd ed. Pp. 883; 1525 illustrations. Philadelphia: Lea & Febiger, 1946. Price, \$12.00.

Exercises in Electrocardiographic Interpretation. By LOUIS N. KATZ, A.B., M.A., M.D., F.A.C.P., Director of Cardiovascular Research, Michael Reese Hospital, Chicago, Ill.; Professorial Lecturer in Physiology, University of Chicago, Chicago, Ill. 2nd ed. Pp. 288; 307 illus. Philadelphia: Lea & Febiger, 1946. Price, \$6.00.

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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

AUGUST, 1946

ORIGINAL ARTICLES

THE LEUKOCYTIC RESPONSE OF PATIENTS WITH EXPERIMENTALLY INDUCED INFECTIOUS HEPATITIS*

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THE leukocytic response of patients with infectious hepatitis has been described as *leukopenia* with a decrease of all the components of the leukocytes, except the monocytes. Some observers⁷ have reported an early transient leukocytosis, with leukopenia becoming evident at the time of the appearance of jaundice; others^{16,18} have stated that leukopenia is an early phenomenon appearing in the pre-icteric period. A frequently observed feature of this disease has been a *relative lymphocytosis* developing subsequent to an early lymphopenia. Of particular interest in this regard has been the appearance of numerous large mononuclear cells defined variously as immature or atypical lymphocytes, or atypical monocytes.^{7,16,18} The association of such cells with the posterior cervical adenopathy, so commonly observed in infectious hepatitis, has been the subject of discussion.¹

Aside from the broad general association of pyogenic infections with leukocytosis and virus infections with leuko-

penia, the pattern of leukocytic response in various acute infections is limited. Quantitative alterations in the various components of the leukocytes, particularly the early leukopenia with neutropenia and lymphopenia, similar to those found in infectious hepatitis, have been observed in several acute infectious diseases caused by rickettsia and viruses.¹⁷ Such changes have been described as occurring in measles,² pappataci fever,¹³ dengue,^{12,14} varicella,⁶ smallpox,¹⁷ yellow fever,³ influenza,¹⁷ rubella,⁹ typhus fever,⁸ and psittacosis.¹¹

Qualitative changes with the appearance of atypical lymphocytes similar to those seen in patients with infectious hepatitis have also been reported as occurring in measles² and German measles.^{2,15} A further comparison of such cells with those ordinarily considered as pathognomonic of infectious mononucleosis² has been made.

During the past 18 months, experiments conducted by the Neurotropic Virus Dis-

* Representing work done for the Neurotropic Virus Disease Commission of the Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, in the Preventive Medicine Service of the Office of The Surgeon General, U. S. Army, Washington, D. C.

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ease Commission, on the transmission of infectious hepatitis to human volunteers¹⁰ have made it possible to study the hematologic response of such patients throughout the incubation period and course of disease. It is the purpose of this paper to report on the leukocytic changes observed in 26 patients during the course of experimentally induced hepatitis. In 10 of these volunteers similar observations were made at regular intervals throughout the period of incubation as well as during the course of disease.

Materials and Methods. *Subjects.* The subjects were previously healthy, male, human volunteers, ranging in age from 19 to 29 years. These men contracted infectious hepatitis experimentally, following inoculation or ingestion of material known to contain the etiologic agent of the disease. Prior to the onset of disease they were housed in 3 groups: in Middletown, Danbury, and New Haven. During the course of illness all patients except those at Danbury were hospitalized in the Isolation Pavilion of the New Haven Hospital. The group at Danbury was hospitalized at that institution. The diagnosis of infectious hepatitis was made on the basis of characteristic symptoms and signs, accompanied by fever and consistent deflection of the bromsulfalein dye retention and cephalin-cholesterol flocculation tests. All patients mentioned in this report had clinical jaundice.

Virus. The strain of virus used in this laboratory was originally obtained from the stool of a U. S. Army soldier (BE), who contracted epidemic infectious hepatitis in Sicily in September, 1943.⁵ It has been through 4 passages in human volunteers to date. This agent is filtrable through an L2 Chamberland filter and withstands heating to 56° C. for at least 30 minutes.⁴ It has produced the disease in 27 out of 40 human volunteers (including those reported here) following parenteral or oral inoculation with incubation periods ranging from 15 to 34 days.

Blood Counts. 600 total leukocyte and differential counts were made on the 26 human volunteers. Blood counts were made between 9 and 10 A.M., after breakfast. The usual method for determination of the total number of leukocytes was employed.

Blood obtained from the finger-tip was diluted in standardized pipettes and shaken for 3 minutes by hand. The number of cells in the 4 corner squares of a standard hemocytometer was counted. Differential counts of fixed smears were made on slides or cover-slips stained with the May-Greenwald-Giemsa technique. 400 to 600 cells were counted and the absolute values were determined. A mechanical stage was used. Care was taken to avoid the periphery of the slides where the smears were very thick and likewise the center of the cover-slips where the greatest pressure has occurred in preparation.

The range and average determinations of the various components of the leukocytes for these 26 human volunteers were computed before the experimental inoculations. Four total counts and differential smears were examined for each man over a period of several days. The results appear in Table 1 and are used as the averages against which the deflections of individual responses during incubation period and course of disease are plotted in the various charts.

TABLE 1.—LEUKOCYTE PICTURE IN 26 HEALTHY HUMAN VOLUNTEERS BEFORE EXPERIMENTAL INOCULATION WITH "VIRUS" OF INFECTIOUS HEPATITIS

Leukocytes	Number / cu.mm.	
	Range	Average
Total	4500-10,900	6700
Neutrophils	1990- 7,400	4100
Seg. neutrophils	1930- 6,400	3650
Non-seg. neutrophils	60- 1,420	450
Lymphocytes	1440- 3,160	2160
Large lymphocytes	120- 980	400
Eosinophils	50- 600	160
Monocytes	90- 490	210
Basophils	0- 180	40

In these experiments leukocyte counts were made every other day throughout the incubation period and almost daily during the first 3 weeks of disease in 10 patients. In the other 16 patients similar studies were made every other day during the first 3 weeks of disease. During the 4th week all 26 patients were examined every 2 to 4 days.

Results. When the leukocytes are counted frequently a regular pattern of response in infectious hepatitis is observed. These characteristic changes usually begin in the first 24 to 48 hours of the acute, febrile, pre-icteric phase of disease

and are primarily associated with the presence of fever. The 26 patients reported here may be almost equally divided into 2 groups. One group had an acute onset of disease with fever. The other group had an insidious onset with vague abdominal symptoms for periods of 2 to 11 days before the appearance of fever. In general, it was observed that the 12 patients who made up the former group manifested the characteristic leukocytic response within the first 24 to 48 hours

minor leukocytic changes in the presence of symptoms 2 to 3 days before fever.

By using the appearance of fever as the fixed point, it is possible to present the combined leukocytic response in all 26 patients of this series. When this is done and the average duration of fever and jaundice, and the appearance time of jaundice are recorded it is seen that defervescence, the appearance of jaundice, and a *beginning* approach to normal leukocytic relationship occur at the end of the 1st

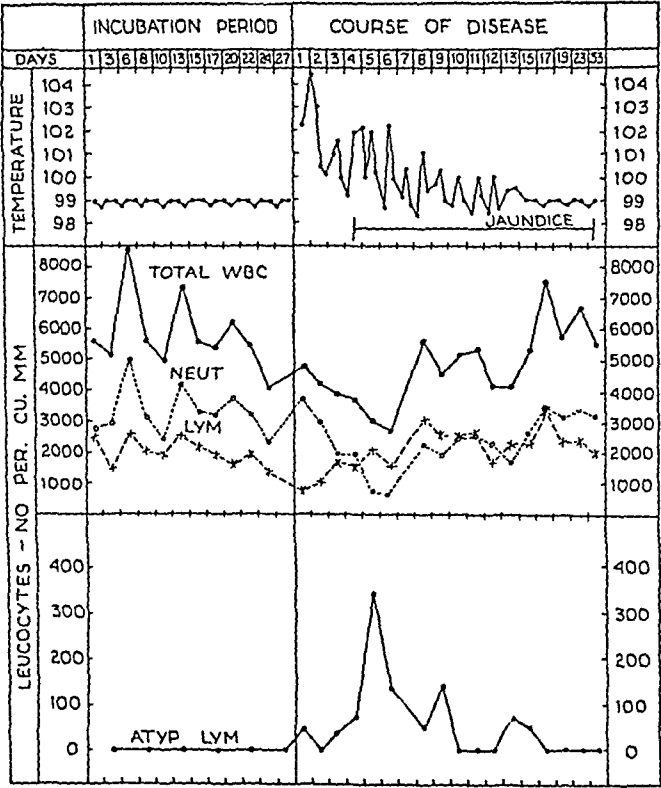


FIG. 1.—Incubation period and course of disease of a patient (SR) with experimentally induced infectious hepatitis. Rectal temperatures are recorded.

of disease, although minor changes might appear in certain individuals in the last 2 to 3 days of the incubation period. (Fig. 1.) In contrast it was evident that the 14 patients in the latter group who had a vague onset with mild generalized or abdominal complaints and a normal temperature usually did not develop the characteristic pattern of change until several days later coincident with the appearance of fever (Fig. 2). However, occasional individuals in this group had

week of fever (Fig. 3). In general, it may be said that the early dramatic changes in leukocytic response (leukopenia, lymphopenia and neutropenia) are almost over by the time jaundice appears, although in some patients in whom jaundice appears as early as on the 3rd to 4th day of fever, many of the striking changes are still present (Fig. 1). In certain individual cases, also, a mild relative lymphocytosis with a small percentage of atypical cells continues during the 2nd week, after

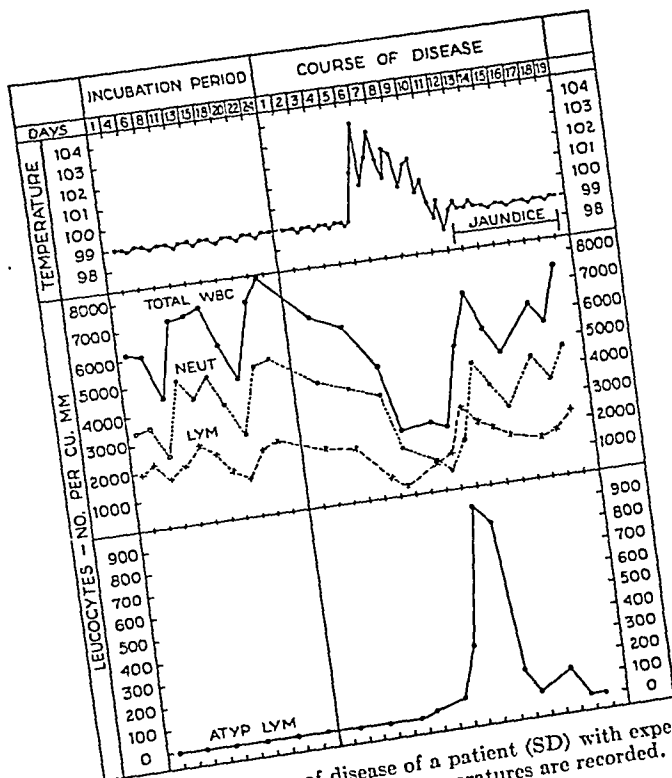


Fig. 2.—Incubation period and course of disease of a patient (SD) with experimentally induced infectious hepatitis. Rectal temperatures are recorded.

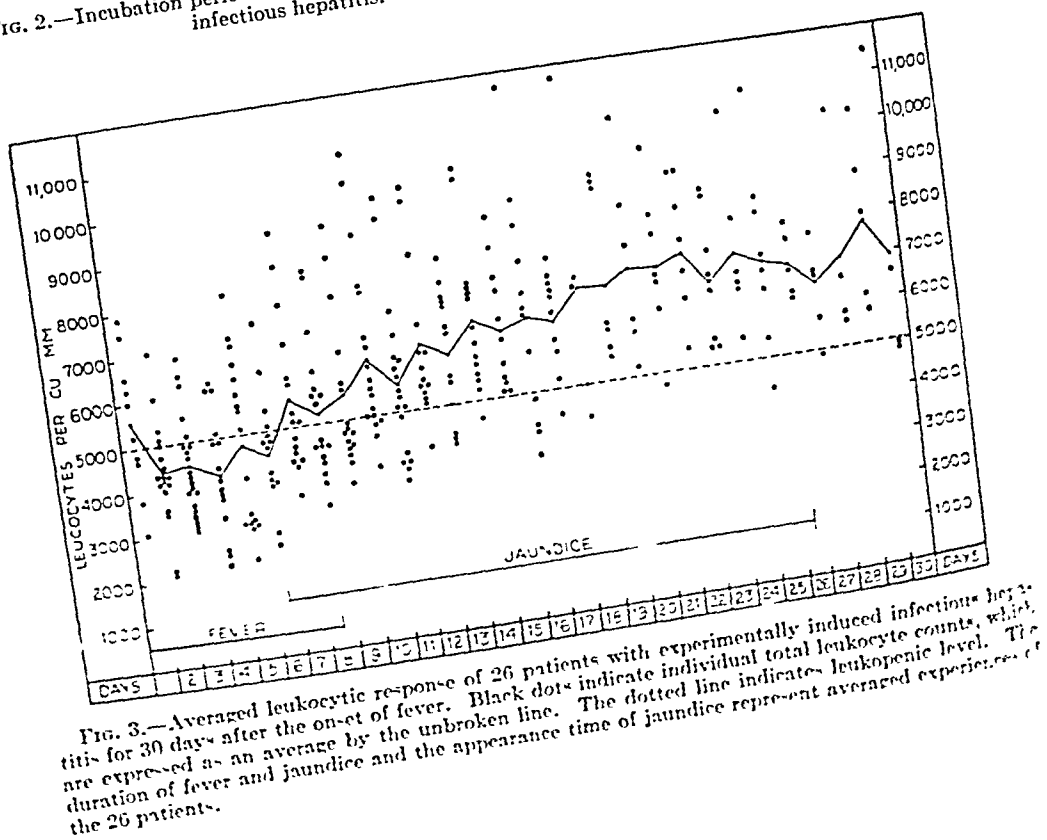


Fig. 3.—Averaged leukocytic response of 26 patients with experimentally induced infectious hepatitis for 30 days after the onset of fever. Black dots indicate individual total leukocyte counts, which are expressed as an average by the unbroken line. The dotted line indicates leukopenic level. The duration of fever and jaundice and the appearance time of jaundice represent averaged experiences of the 26 patients.

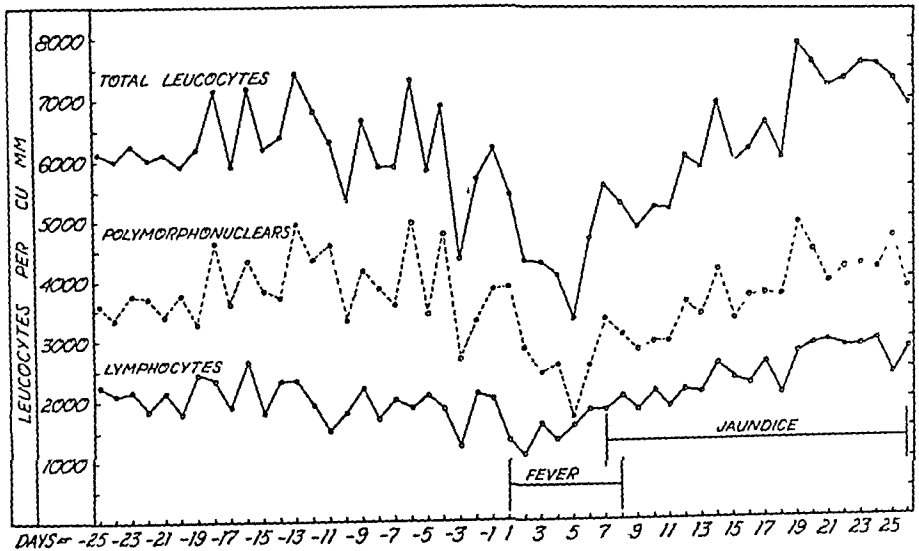


FIG. 4.—Averaged leukocytic response of 10 patients during the incubation period and course of disease of experimentally induced infectious hepatitis. (—) indicates days before onset of fever. The duration of fever and jaundice and the appearance time of jaundice represent averaged experiences of the 10 patients.

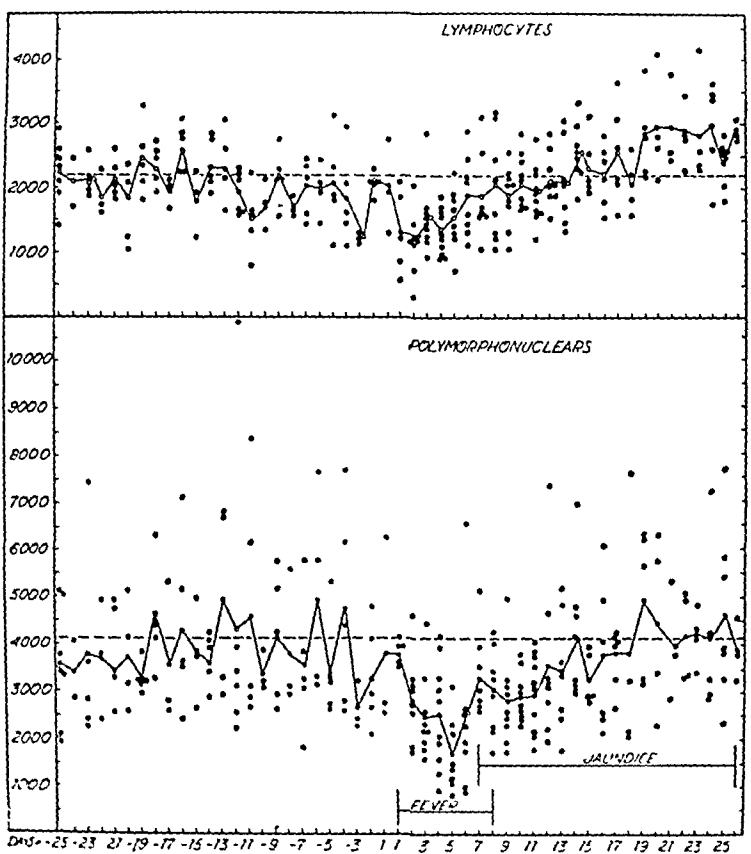


FIG. 5.—Leukocytic response of 10 patients during the course of disease of experimentally induced infectious hepatitis. (—) indicates days before onset of fever. The duration of fever and jaundice and the appearance time of jaundice represent averaged experiences of the 10 patients. Black dots indicate individual determinations which are expressed as an average by the unbroken line. Dotted lines indicate the average normals for the group.

the appearance of jaundice, and in an occasional case a secondary dromedary type of curve similar to the first response occurs in the 2nd or 3rd week (Fig. 1).

For purposes of convenience the leukocytic changes are described as occurring in 3 phases, namely, (1) the incubation (pre-febrile) phase, (2) the febrile (pre-icteric) phase and (3) the icteric (post-febrile) phase.

Incubation (Pre-febrile) Phase. When incubation periods were measured from the day of inoculation to the first appearance of symptoms they ranged from 15 to 28 days. However, the time interval from day of inoculation to *onset of fever* in these patients ranged from 24 to 30 days. Using the onset of fever as a fixed point, the averages of the total number of leukocytes and their individual components were computed for 25 days before fever and 26 days after fever in 10 patients. (Figs. 4 and 5.)

In general there is little fluctuation from normal during the pre-febrile period up until 2 to 3 days before the onset of fever. During the 4th week, before fever, the total number of leukocytes is slightly below the normal average for the group (6700 per c.mm.). Both neutrophils and lymphocytes are diminished in proportion. In the 3rd week, before fever, the total leukocyte count is slightly above normal limits and there is a slight average increase of neutrophils with a slight decrease in lymphocytes. This average increase of total leukocytes is maintained during the 2nd week, along with an increase in neutrophils and decrease of lymphocytes. *Three days* before the appearance of fever the total number of leukocytes drops below the established normal for this group (6700 cells per c.mm.). At this time total counts as low as 4000 cells per c.mm. occasionally occur. This decrease in absolute numbers is shared proportionately by the neutrophils, eosinophils, basophils and lymphocytes. The monocytes are not affected. Such leukopenia before the onset of fever may or may not be accompanied by symptoms, depending on

whether it occurs in a patient with an acute or insidious onset of disease.

Febrile (Pre-icteric) Phase. The duration of fever ranged from 4 to 14 days, averaging 8 days. Clinical jaundice ranged in appearance time from 3 to 12 days after the beginning of fever, averaging 6 days (Fig. 3). In all but 1 patient leukopenia, *i. e.*, a reduction to below 5000 leukocytes per c.mm., was found at some time during this febrile pre-icteric phase of disease. The total number of leukocytes ranged between 2250 and 9400 per c.mm. Characteristically leukopenia occurred early, appearing in 25 patients during the 1st 24 to 48 hours of fever, becoming more pronounced until the 4th or 5th day when the low level was reached. The total number of leukocytes began to increase through the 5th and 6th days, rising above the level of leukopenia on the 7th day.

Associated with the leukopenia are certain quantitative changes in the various components of the leukocytes.

Neutrophils. On the 1st day of fever there is a *relative* increase in neutrophils in the presence of leukopenia or low-normal total leukocyte count. Although the absolute number of neutrophils is less than normal, this *relative* increase is possible because of a coincident lymphopenia. By the 2nd day absolute neutropenia is present and this progresses, reaching a low point on the 5th day of fever. Absolute numbers as low as 820 cells per c.mm. has been recorded. On the 6th and 7th days the neutrophils rise sharply although they do not reach the normal level for the group. (Figs. 4 and 5.) During the 1st 5 days of fever, as neutropenia increases, the non-segmented cells increase somewhat in absolute value, but due to the marked decrease in segmented cells, they may number as many as 50% of the total neutrophil count. After the 5th day this relationship is changed and the absolute value of segmented cells rises sharply on the 6th and 7th days, so that the mature cells become again predominant. (Fig. 6)

Eosinophils. Eosinophilic granulocytes are diminished in absolute numbers during the acute febrile phase of the disease. They are below normal on the 1st day of fever, decline to a low level on the 3rd day, which is maintained until the 5th day after which a slight increase occurs on the 6th to 8th day, although a normal level is not reached.

Basophils. The change in absolute numbers of basophils is not great, but these cells are diminished during the febrile phase.

Monocytes. The absolute numbers of monocytes are sharply depressed during the febrile phase.

The lymphopenia of the 1st 5 days of the febrile period is largely at the expense of the small round cells. The large lymphocytes increase somewhat during this period and, because of the decrease in small cells, may form as many as 40 to 70% of the total number of lymphocytes. Characteristically, on the 1st or 2nd day of fever a few of these large lymphocytes are atypical in appearance. These atypical cells increase in number so that by the 5th day a majority of the large cells may be atypical (Figs. 1 and 2). It is at this time when there may be neutropenia and *relative* lymphocytosis with a predominance of large atypical

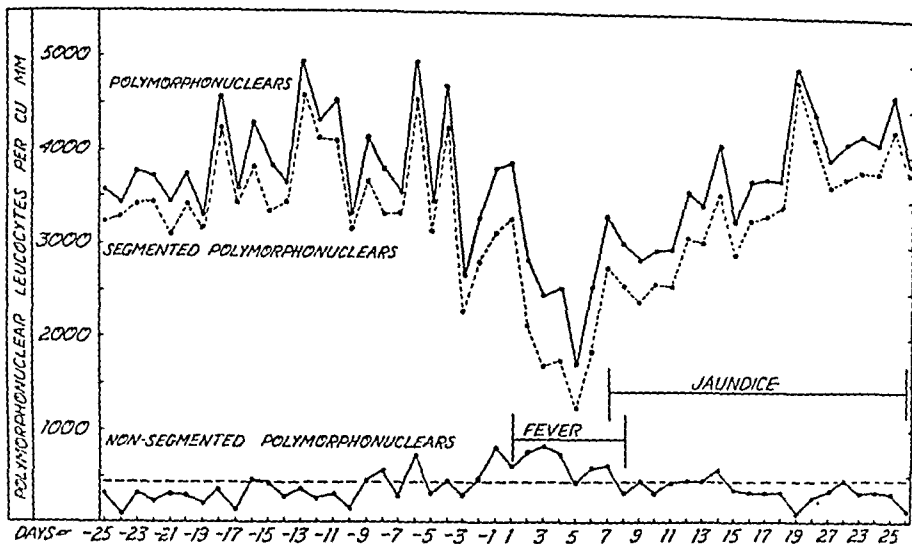


FIG. 6.—Averaged polymorphonuclear leukocytic response of 10 patients during the incubation period and course of disease of experimentally induced infectious hepatitis. (—) indicates days before onset of fever. The duration of fever and jaundice and the appearance time of jaundice represent averaged experiences of the 10 patients. Dotted line indicates average normal level of non-segmented polymorphonuclears for the group.

Lymphocytes. On the 1st day of fever both absolute and relative lymphopenia is present. This reaches a low point on the 2nd day, persisting at a low level through the 4th day. By the 5th day when neutropenia is most marked, the lymphocytes begin to increase, equalling or outnumbering the neutrophils and resulting often in a relative lymphocytosis. An absolute lymphocytosis rarely occurs. The absolute number of lymphocytes continue to rise through the 6th to 8th day although a normal level is not reached.

lymphocytes that considerable difficulty may be encountered in differentiating this leukocytic response from that found in infectious mononucleosis. This is particularly true when the posterior cervical lymphadenopathy, as commonly observed in this phase of infectious hepatitis is present,¹ and jaundice has not yet appeared. From the 5th to the 7th days the number of small lymphocytes increases and they again become predominant.

These atypical cells may be described as large lymphocytes with big eccentric

cally placed indented nuclei. At times an area of hyaloplasm containing an azure granule is seen at the indentation of the nucleus. The cytoplasm is usually heavily basophilic and the chromatin of the nuclei

have heavy blocks of chromatin in the nuclei. (Fig. 7.)

Icteric (Post-febrile) Phase. The duration of jaundice ranged from 8 to 31 days, with an average of 20 days. Character-

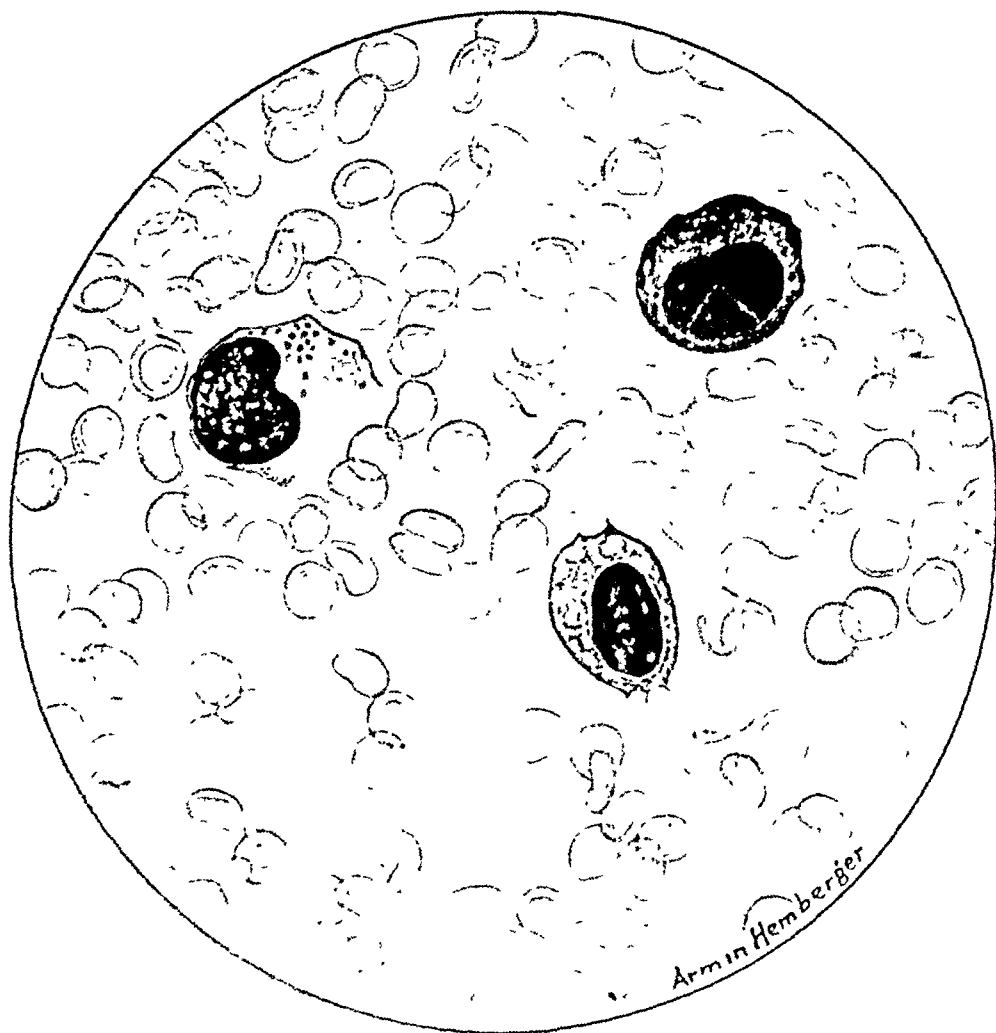


FIG. 7.—Lymphocytes found in the blood on the 5th day of fever of a patient with experimentally induced infectious hepatitis. (Photograph of a colored drawing by Armin Hemberger.) Giemsa-May-Greenwald stain. ($\times 1125$.)

is arranged in heavily staining bands. Vacuoles are frequently found in both the cytoplasm and nuclei. Other large atypical cells may have a paler blue staining cytoplasm containing numerous coarse, azurophil granules. Such cells frequently

istically, clinical jaundice appears with defervescence at the end of the 1st week. At this time the trend of the leukocytic response is in the direction of normal relationships. During the 2nd week the total number of leukocytes rises gradually,

reaching the average normal level of the group on the 14th day (Figs. 3 and 5). This increase usually reflects a proportional increase in all the various components of the leukocytes although important variations may occur. Post-febrile leukocytosis as high as 12,500 cells per c.mm. has been seen. Not infrequently a relative lymphocytosis may persist during the 2nd week with a small proportion of large atypical cells evident, although the small lymphocytes are predominant. Also, on occasion, a secondary "camel" type of curve of lesser degree but resembling the acute changes found in the 1st week may occur (Fig. 1). During the 3rd and 4th week as jaundice reaches its peak, wanes and disappears, the number of leukocytes and the relations of their various components is usually within normal limits.

Discussion. It has been pointed out in this paper that a regular pattern of leukocytic response occurs during the course of infectious hepatitis *experimentally induced* in human volunteers. This is in general agreement with the findings of others working with the naturally occurring disease and quite similar to the response observed in a number of acute infectious diseases caused by viruses and Rickettsia. The latter point may be of some importance as a further bit of evidence for the classification of infectious hepatitis as a disease of "virus" etiology.

It is of interest to note that although slight changes in the leukocytes may occur in individual patients in the last 2 to 3 days before the onset of fever, the characteristic leukopenic response is related primarily to the febrile, pre-icteric phase. Jaundice, which occurs at the end of the 1st week is associated in general with defervescence and a beginning return to normal leukocytic relationships. This is analogous to the situation in measles in which similar changes have been observed associated with the appearance of fever.²

The similarity between certain atypical lymphocytes found in patients with infectious hepatitis, measles, German measles, and infectious mononucleosis has been indicated. The fact that such similar cells appear in several diseases of different etiology suggests that their production represents a limited response to a broad group of acute infectious diseases. This is of some practical importance in differential diagnosis, particularly as it concerns infectious hepatitis and infectious mononucleosis. Not infrequently the appearance of a large percentage of atypical lymphocytes in a patient during the febrile, pre-icteric phase of infectious hepatitis causes confusion. This is particularly true when there is an associated enlargement of the posterior cervical glands.

Summary. 1. The results of serial observations on the leukocytic response of patients during the incubation period and course of disease of *experimentally induced* infectious hepatitis are presented.

2. Although minor changes in leukocytes may occur in the last 2 to 3 days before the onset of fever, the characteristic leukocytic response begins in the first 24 to 48 hours of fever and is associated primarily with the febrile, pre-icteric phase of disease.

3. The pattern of this leukocytic response is characterized by the early appearance of leukopenia: lymphopenia and neutropenia. Subsequent relative lymphocytosis with numerous atypical lymphocytes is a common phenomenon.

4. Defervescence, the appearance of clinical jaundice and the trend towards normal leukocytic relationships are commonly associated with the end of the 1st week of fever.

5. The return to normal leukocytic relationships is completed by the end of the 2nd week after the appearance of fever.

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RETROPERITONEAL SPLENOMEGALY

OCCURRENCE IN A CASE OF LEUKOPENIC PLASMA CELL LEUKEMIA

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IN contrast to the enormous literature on the wandering or movable spleen, that on the retroperitoneal spleen is extremely meager. The reasons for this are obvious: the movable spleen is not rare and is easily felt on palpation, although many instances are recorded of its being mistaken for other intra-abdominal masses, even for a pelvic tumor or a pregnant uterus. Also, the movability itself tends to splenic enlargement from congestion and even torsion of the lengthened pedicle may occur. A wholly retroperitoneal position of the spleen is apparently very rare, although the paucity of symptoms and difficulty of recognition may enter into this view.

Developmentally, the spleen originates in the posterior mesogastrium and acquires a complete peritoneal covering. An eventual retroperitoneal position is a distinct developmental abnormality. In the retroperitoneal position the anatomic relations of the organ differ from those found in the normal in that it tends to rest upon the upper pole of the left kidney and posterior to the colon. This relationship to the kidney may explain one of the disorders seen in individuals with the spleen in the retroperitoneal position; that to the colon contributes to the difficulty of identification by physical examination.

The occasional location of the spleen in a retroperitoneal position is mentioned in many books on anatomy and medicine; but a careful search of the literature reveals practically no references to this subject and no report of a case such as is to be detailed here. Spencer² has reported a "Case of retroperitoneal prolapse of the spleen into the left loin." The pa-

tient, a woman of 32 years, had suffered 9 attacks of pain in the left loin with vomiting, and had the feeling that in that region a "lump" came and went. On examination, there was found deep tenderness and a mass behind the colon. It was noted that normal splenic dullness was not present. The Roentgen ray revealed the kidney and a shadow above it, which was thought to be a hydronephrotic upper pole. Intending to expose the kidney, the surgeon came upon the spleen. This was removed and was found to be 2 or 3 times the normal size. After the splenectomy it was seen that the kidney had been pushed downward by the overlying spleen. No explanation of the symptoms nor the enlargement of the spleen was ventured, no postoperative history given.

Two somewhat similar patients are reported by Ehrich,¹ in each of whom there was left hydronephrosis. At operation, the spleen was found behind the peritoneum in a dense common capsule with the kidney. These cases seem to suggest that hydronephrosis may be associated with a retroperitoneal position of the spleen, perhaps as a result of displacement of the kidney downward with kinking of the ureter.

In the unique case now to be recorded, the diagnosis of an unusual type of leukopenic (aleukemic) leukemia was made more difficult by the presence of a large mass in the left renal region which defied identification as spleen—a fact which was explained at the autopsy when an enlarged retroperitoneal spleen was found.

Case Report (Hosp. U. of Pa. 44-68456). The patient, a male of 63 years, commenced

to feel fatigued and weak 4 years before admission on Oct. 3, 1944. Six months before this date, he received, in another hospital, 20 blood transfusions for anemia and further transfusions recently in the same institution. He was carefully studied there and the bone marrow was found to be infiltrated with large (possibly malignant) unidentifiable cells. No primary neoplasm could be found and the final diagnosis was aplastic anemia (etiology unknown). In the month before admission, he lost weight rapidly and had irregular fever. Except for diabetes mellitus his past medical history was negative.

The patient's physical condition was very poor with evident pallor and emaciation. A slight but widespread enlargement of palpable lymph nodes was present. On inspiration the liver edge was felt 5 cm. below the rib margin. In the left kidney region, the kidney could be identified, and, with difficulty, another mass could be felt lying more posteriorly than anteriorly and not obliterating the tympany of Traube's semilunar space. This mass presented no edge and only came down about 2 fingers' breadth below the costal margin on deep inspiration. On the skin of the thigh were several indurated, palm-sized plaques which were diagnosed as areas of leukemic infiltration by the dermatologic consultant.

Of the extensive laboratory studies, only the following need be detailed: in the peripheral blood, a severe normocytic anemia with the erythrocyte count varying a little above or below 2,000,000 and the leukocytes ranging between 1000 and 3000. There was a reduction in neutrophils and a resulting high percentage of lymphocytes. No abnormal or immature forms of white blood cells were seen until terminally. Three plasma cells were noted on 1 occasion. The thrombocyte count varied from a normal to a low level. A careful study of the bone marrow obtained by sternal puncture led, for the second time, to the opinion that the numerous large, vesicular, nucleated cells were evidence of malignant infiltration and that the process was not leukemia. The usual evidence of mild diabetes mellitus was present.

Pursuing the above suggestion, a second search for a primary neoplasm was carried out. Roentgenograms of chest, gastro-intestinal tract, urinary tract, and most of the skeleton revealed nothing abnormal except confirmatory evidence that there was a large

abnormal mass in the left kidney region pushing the diaphragm up and the kidney down (Fig. 1). In consultation with the roentgenologists, they pointed out that if this mass were spleen it should be more easily palpable; that it lay above the kidney pushing the diaphragm upward. The suggestion was made that it might be a retroperitoneal neoplasm such as hypernephroma.

In addition to his progressive anemia and wasting, the patient had a mild diabetes which was controlled with 10 units of insulin daily. He also had many subcutaneous abscesses requiring drainage. These infections and his poor general condition made biopsy of a lymph node unwise. In spite of transfusions, penicillin and general care, the patient failed rapidly and died on Nov. 25, 1944, after 7 weeks stay in the hospital.

The diagnostic problem presented by the patient was an interesting one. It was believed that the presence of diabetes was a coincidence and could be disregarded. Essentially the diagnosis rested on the identification of the abnormal cells in the bone marrow and of the abnormal mass above the left kidney. The anemia could be explained by the heavy infiltration of the marrow with abnormal cells, whether of neoplastic or leukemic nature. Several pathologists and hematologists agreed that these cells were malignant and not leukemic. If this opinion were correct, it must argue strongly for the neoplastic nature of the mass above the left kidney. If, however, the abnormal cells were leukemic, then an enlarged spleen might well be anticipated. The mass, however, did not offer the usual criteria for recognition of an enlarged spleen. No edge or notch could be felt; it was not in the usual position, it did not obliterate Traube's area of tympany, the colon lay in front of it and the kidney was pushed down by it. These were our views at the time of the patient's death; no one had suspected a retroperitoneal splenomegaly.

Autopsy. (J. Snyder—7 hours after death.) As is so often the case in the elderly, there were present many pathologic findings which apparently bore no immediate relation to the disease causing death. In this 63 year old asthenic individual, there were found a dissecting aneurysm of the aorta, thrombosis in the right auricle and venacava, nephrosclerosis and necrosis of the esophageal mucosa. The pertinent findings in-

cluded widespread leukemic infiltration of bone marrow, lymph nodes, liver and spleen with cells of the plasma cell variety. This clinched the primary diagnosis and leaves for discussion the abnormal mass in the left kidney region. This mass proved to be

abnormality of the kidney. Under the microscope, it was seen that "the normal architecture of the spleen has been definitely disrupted and the spleen tissue consists merely of a diffuse cellular tissue showing a majority of plasma cells with lympho-

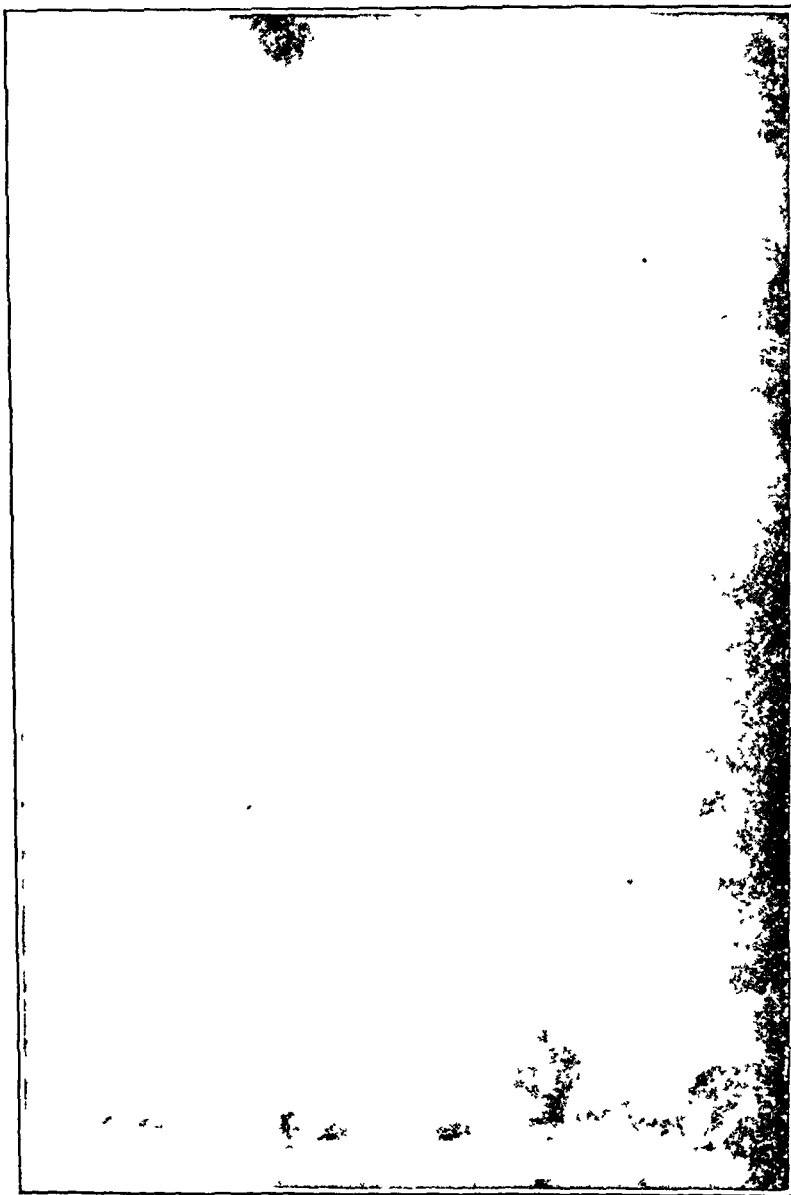


FIG. 1.—Film from urogram series showing enlarged retroperitoneal spleen pressing down on left kidney.

a large completely retroperitoneal spleen, weighing 650 gm., enclosed in a well-fitted peritoneal capsule. It was somewhat oval in shape, bluish red in color and increased in consistency. Although the left kidney was pushed down by the spleen, there was no evidence of hydronephrosis or any gross

cytes seen in the minority." This same picture was seen in the lymph nodes and bone marrow and to a lesser degree in the liver.

Discussion. From this brief review of the literature and this case report, it would

seem that the following statements can be made: (1) The spleen, as a result of a rare developmental anomaly may occupy a wholly retroperitoneal position. (2) The retroperitoneal spleen may cause no symptoms but occasionally may be associated with left hydronephrosis and may perhaps cause this condition by displacement of the kidney downward. (3) A retroperitoneal spleen may be enlarged in any of the diseases which cause splenomegaly; when

this occurs the resulting mass is difficult to identify by physical examination or roentgenography. (4) The enlarged retroperitoneal spleen pushes the left diaphragm upward and the left kidney downward; it lies posterior to the stomach and the colon and does not obliterate the tympany of Traube's semilunar space; it fails to present to palpation the characteristic splenic edge or notches.

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A MODIFIED CEPHALIN CHOLESTEROL TEST (HANGER) IN THE STUDY OF HEPATIC DISEASE*

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As devised by Hanger,^{4,5} the cephalin cholesterol flocculation test has proved to be a valuable tool in the study of liver disease. There are, however, certain inherent difficulties in the procedure which have led to irregular results.^{2,3,9,12,13,14,16} Thus, if ripe cephalin was utilized, false negative tests were obtained, whereas "ripening" of the cephalin by exposure to sunlight for a few weeks caused false positive reactions.^{9,15} Further investigation⁹ revealed that prolonged exposure of the antigen to sunlight eliminated some flocculation with normal sera. Apparently the ripening process tends at first to increase markedly the cephalin sensitivity, and later to decrease it for some reason or other.⁸ The effect of variability in sensitivity of the emulsion on the final result is illustrated by the recent report of Lippincott *et al.*,⁷ who found that tests with serum from acute cases of relapsing vivax malaria yielded 82% positive reactions with one cephalin cholesterol emulsion, whereas tests with another antigen were positive with only 21% of the same sera. The Hanger procedure is further complicated by the fact that fresh sera must be used,⁵ preferably samples drawn within 6 hours of testing.^{2,14,16} More recently the studies of Neefe and Reinhold,¹² and also Mateer⁸ have emphasized photosensitivity as a source of some false positive reactions. Thus normal serum antigen mixtures exposed to sunlight or

to an incandescent lamp for a period of 48 hours almost invariably flocculated while duplicate specimens kept in the dark remained in homogeneous suspension. They¹² further found that if normal serum was permitted to stand diluted for 5 or more hours prior to the addition of emulsion, flocculation occurred. Conducting the test at 37° C. resulted, in some instances, in flocculation although no reaction was noted at 21° C.^{2,12}

The objective of the present investigation has been to obtain a stable but sensitive sterol emulsion which could be utilized for the examination of serum in a manner similar to that used in serologic tests.

Methods. *Preparation of Hanger Antigen (H).* Thirty-five ml. of redistilled water were heated to 70° C. in a 150 ml. Erlenmeyer flask. One ml. of stock etherized cephalin cholesterol mixture (Difco)† was added dropwise and with agitation. The emulsion was then permitted to boil slowly until the total volume had been reduced to 30 ml., gradually cooled to room temperature, and utilized in the test.^{5†}

Preparation of the Modified Antigen (M). One ml. of stock etherized cephalin cholesterol mixture (Difco) was added dropwise and with agitation to 35 ml. of redistilled water at room temperature (20° to 25° C.). The emulsion was then permitted to boil slowly until the total volume had been reduced to 30 ml., gradually cooled to room temperature, and utilized in the test.

The 2 antigens differed considerably in

* These investigations were aided through the Commission on Influenza, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon General, U. S. A.

† Reduction in volume of the emulsions was accomplished by placing the flasks on a hot-plate and boiling gently for 6 minutes at 550° F.

‡ Furnished through the courtesy of the Difco Laboratories, Inc.

appearance and in reaction. The new preparation is more opalescent and the particles are more coarsely dispersed; furthermore, serum sterol mixtures produced a granular precipitate rather than the flocculent type usually obtained with the Hanger antigen.

Performance of the Standard Test. Two-tenths ml. of serum was added to 4 ml. of 0.85% saline. Shortly thereafter, 1 ml. of cephalin cholesterol antigen was added, the reagents were thoroughly mixed, placed in the dark room at 20° to 25° C., and examined in subdued light at the end of 24 and 48 hours. Readings from 0 to 4+ were recorded after the method of Hanger.⁵

Performance of Titration. Serial dilutions of serum in 0.85% saline were made in chemically clean serologic tubes as follows: 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256. A single pipette was used for the entire series of dilutions and the final volume in each tube was 0.8 ml.; 0.2 ml. of the modified antigen was then added to all tubes. The mixtures were shaken thoroughly and then placed in the ice-box at 5° C. unless otherwise stated. They were examined 18 to 20 hours later. The intensity of the reaction was indicated as follows: 1+, a readily visible granularity of the emulsion; 2+, an increased granularity with or without slight sedimentation; 3+, heavy agglutination of the particles accompanied by 50 to 75% sedimentation; 4+, complete agglutination and sedimentation of the emulsion. Occasionally, with normal sera the tubes containing the 1:4 and 1:8 dilutions showed simple sedimentation without the characteristic conglomeration of particles when the settled material was stirred up. Such sediments were classed as negative reactions. All titration experiments were carried out with the M antigen unless otherwise stated.

Interpretation of Titration Test. The degree of flocculation in each tube of the serial dilution was recorded after incubation at 5° C. for a period of 18 to 20 hours. The total number of plusses was then added and constituted a cumulative score. The maximum score for a 7 tube titration was 28, provided all tubes showed 4+ flocculation. Conversely, the minimum score was 0. For best interpretation of results the degree of flocculation in each tube should be reported together with the cumulative score. For this purpose a graph can be readily constructed.

Results. *Influence of Ageing, Light and Dilution Upon Reactions With Normal Sera.* Three sets of comparative data with sera from 24 individuals who were considered to be entirely well are presented in Table 1. In Experiment A the standard flocculation test was performed with the Hanger antigen and again with the modified cephalin cholesterol emulsion under favorable conditions, namely, fresh, undiluted sera which had been protected from light at all times. One + reactions with either antigen were considered to be within normal limits. Sera 5 and 7 showed 2+ flocculation with the Hanger antigen after 48 hours incubation at room temperature. No significant flocculation occurred with the new antigen under the same conditions.

In Experiment B the sera were diluted and permitted to stand in the sunlight for 6 hours prior to the addition of the lipid emulsions. Using Hanger antigen, within 24 hours 2+ or greater flocculation was evident in 8 specimens and by 48 hours the number had increased to 12. In contrast the modified antigen remained in homogeneous suspension with all 24 sera.

In Experiment C the same sera were retested after storage at 4° to 5° C. for a period of 6 months. By this time 21 specimens gave positive flocculation with Hanger antigen in 24 hours and all but 1 reacted within 48 hours. The altered antigen, however, showed significant flocculation only with Serum 16 at 24 hours and also with Sera 20 and 21 after 48 hours incubation.

Simultaneously the same sera were tested by the serial dilution method in Experiment D. The highest score recorded was 7+ in 2 instances, whereas the serum from a case of acute hepatitis showed a score of 23. These data confirmed the previous observation that the cephalin cholesterol flocculation test performed according to the method of Hanger was not reliable with diluted and light exposed, or with old sera.^{11,12} They showed, on the other hand, that with the modified antigen these false positive reactions with normal sera were essentially eliminated.

ed, particularly if the titration technique was utilized.

Influence of Temperature of Incubation on Titration Tests With Normal and Abnormal Sera. The effect of varying the temperature of incubation on the serial dilution test is presented in Table 2. Incubation at 5° C. and at 21° C. resulted in strong flocculation with sera from 2 cases of acute hepatitis and weak or no reactions with normal samples. When the temperature was elevated to 37° and 56° C., the differences between hepatic and normal sera were greatly decreased.

and 15. Acute hepatic sera were usually characterized by the fact that they caused flocculation throughout the entire range of titration at both incubation temperatures. This type of reaction was not entirely limited to hepatitis but also occurred with some sera from other forms of liver disease (Serum 5). The most common pattern seen in convalescent hepatitis, cirrhosis and tumors involving the liver parenchyma was exemplified by Serum 4, namely strong flocculation with serum of low dilution, a narrow zone in which the mixtures remained in homogene-

TABLE 1.—EFFECT OF AGE, LIGHT AND DILUTION ON COMPARATIVE CEPHALIN-CHOLESTEROL FLOCCULATION TESTS WITH NORMAL SERA

Experiment:		A				B				C				D	
Antigen:		Hanger		Modified		Hanger		Modified		Hanger		Modified		Modified	
Incubation hours:		24	48	24	48	24	48	24	48	24	48	24	48	20	
Serum	1	0	0	0	0	3	4	0	0	2	4	0	1	0	
	2	0	0	0	0	3	4	0	0	3	4	0	0	0	
	3	0	0	0	1	0	1	0	1	4	4	0	0	4	
	4	0	0	0	0	0	0	0	0	2	3	0	0	4	
	5	0	2	0	0	0	0	0	0	3	4	0	0	1	
	6	0	1	0	0	3	4	0	0	4	4	0	0	0	
	7	0	2	0	0	1	3	0	0	4	4	0	1	2	
	8	0	0	0	1	2	3	0	0	0	0	0	1	4	
	9	0	0	0	0	0	0	0	0	4	4	0	0	1	
	10	0	0	0	0	1	2	0	0	4	4	0	0	0	
	11	0	0	0	0	2	3	0	0	2	4	0	0	1	
	12	0	0	0	0	0	0	0	0	3	4	0	0	0	
	13	0	0	0	0	0	0	0	0	3	4	0	0	5	
	14	0	0	0	0	2	3	0	0	4	4	0	0	4	
	15	0	0	0	0	0	0	0	0	1	2	0	0	1	
	16	0	0	0	0	0	0	0	0	4	4	4	4	1	
	17	0	0	0	0	0	0	0	0	4	4	0	0	5	
	18	0	0	0	0	1	2	0	0	1	3	0	1	4	
	19	0	0	0	0	0	0	0	0	2	4	0	0	0	
	20	0	0	0	1	0	0	0	0	3	4	1	2	5	
	21	0	1	1	1	2	3	0	0	4	4	1	2	7	
	22	0	0	0	0	1	1	0	0	4	4	0	0	3	
	23	1	1	0	0	2	2	0	0	4	4	0	0	7	
	24	0	0	0	0	1	2	0	0	4	4	0	0	3	
Acute hepatitis:		4	4	3	4	4	4	4	4	23	

A = Fresh sera, light protected and undiluted.

B = Diluted and exposed to sunlight for 6 hours.

C = Retested after standing at 4° to 5° C. for 6 months.

D = Tested by the titration method.

The numbers indicate the degree of flocculation except in Experiment D where they represent the cumulative score after 20 hours incubation at 5° C.

A number of freshly drawn normal and abnormal sera were then examined utilizing 5° and 21° C. as incubation temperatures. These data are shown in Table 3. It was noted that there were 2 zones of reaction, each of which was accentuated by the particular temperature of incubation. Both with normal and abnormal specimens 5° C. favored flocculation with serum of lower dilutions, while 21° C. resulted in an increased flocculation in the higher dilutions of serum as illustrated by Specimens 6, 8, 9, 10, 11, 12, 13, 14,

ous suspension or were only partially flocculated, followed by an end-zone where stronger reactions again appeared. Normal sera differed in that they showed weak or no reactions when diluted 1 to 10, a broad mid-zone in which flocculation was absent, and an end-zone characterized by slight flocculation after 48 hours at 5° C. which was accentuated by incubation at 21° C.

Standard tests with the Hanger antigen as shown in Table 3 indicated that 8 of 10 patients with hepatic disturbance had

521 - 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000, 1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023, 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1034, 1035, 1036, 1037, 1038, 1039, 1040, 1041, 1042, 1043, 1044, 1045, 1046, 1047, 1048, 1049, 1050, 1051, 1052, 1053, 1054, 1055, 1056, 1057, 1058, 1059, 1060, 1061, 1062, 1063, 1064, 1065, 1066, 1067, 1068, 1069, 1070, 1071, 1072, 1073, 1074, 1075, 1076, 1077, 1078, 1079, 1080, 1081, 1082, 1083, 1084, 1085, 1086, 1087, 1088, 1089, 1090, 1091, 1092, 1093, 1094, 1095, 1096, 1097, 1098, 1099, 1100, 1101, 1102, 1103, 1104, 1105, 1106, 1107, 1108, 1109, 1110, 1111, 1112, 1113, 1114, 1115, 1116, 1117, 1118, 1119, 1120, 1121, 1122, 1123, 1124, 1125, 1126, 1127, 1128, 1129, 1130, 1131, 1132, 1133, 1134, 1135, 1136, 1137, 1138, 1139, 1140, 1141, 1142, 1143, 1144, 1145, 1146, 1147, 1148, 1149, 1150, 1151, 1152, 1153, 1154, 1155, 1156, 1157, 1158, 1159, 1160, 1161, 1162, 1163, 1164, 1165, 1166, 1167, 1168, 1169, 1170, 1171, 1172, 1173, 1174, 1175, 1176, 1177, 1178, 1179, 1180, 1181, 1182, 1183, 1184, 1185, 1186, 1187, 1188, 1189, 1190, 1191, 1192, 1193, 1194, 1195, 1196, 1197, 1198, 1199, 1200, 1201, 1202, 1203, 1204, 1205, 1206, 1207, 1208, 1209, 1210, 1211, 1212, 1213, 1214, 1215, 1216, 1217, 1218, 1219, 1220, 1221, 1222, 1223, 1224, 1225, 1226, 1227, 1228, 1229, 1230, 1231, 1232, 1233, 1234, 1235, 1236, 1237, 1238, 1239, 1240, 1241, 1242, 1243, 1244, 1245, 1246, 1247, 1248, 1249, 1250, 1251, 1252, 1253, 1254, 1255, 1256, 1257, 1258, 1259, 1260, 1261, 1262, 1263, 1264, 1265, 1266, 1267, 1268, 1269, 1270, 1271, 1272, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1

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1. The first of these is the fact that the Government has not been able to secure the necessary funds to carry out its policy. This is due to the fact that the Government has not been able to secure the necessary funds to carry out its policy.

[illegible]

All - Acute hepatitis,
Carcinoma of bi

AH = Acute hepatitis.
 CB = Carcinoma of breast.
 CH = Cholestatic hepatitis.

CH - Convalescent
Numbers from 0 to 4 indicate
of hour reading.

- 24 hour reading.
- 48 hour reading.

1451000

C = Cholesterol
AC = Alcoholic extract
Vern = Vernonia

PA = Permeation
of flocculation.

of floor in 1941

CK = Carcinoma of Kidney.
N = Normal.

parenchymatous involvement. The modified antigen seemed much more discriminating when tested by the same method. Flocculation within 24 hours was limited to sera from the 2 patients with acute hepatitis and to 1 with hepatic metastases. After 48 hours 2 additional positive reactions appeared, 1 with serum from a case of convalescent hepatitis and the other from a patient with alcoholic cirrhosis of the liver. These results are presented primarily to illustrate the effect of temperature over a wide range of dilutions which differ from those employed in the titration test as adopted for routine use.

5° C., flocculation in the fore-zone again became evident although it remained somewhat reduced in the sera heated for 30 or 60 minutes.

Influence of Different Lots of Antigen. The effect of varying the commercial lot of cephalin cholesterol on the titration test is given in Table 5. Within the limits of sensitivity of any given antigen the ability to flocculate appeared to be dependent upon the characteristics of each individual serum. Thus, Emulsion E which showed the strongest reactions throughout, gave relatively weak flocculation with Serum 3 and Serum 7 in which the least reactions

TABLE 4.—EFFECT OF INACTIVATION OF SERUM AT 56° C. ON TITRATION TEST

Inactivation 56° C.	Serum	Serum dilutions 1						
		4	8	16	32	64	128	256
None	AH	3*	4	4	4	4	4	4
		4†	4	4	4	4	4	4
	CAV	2	2	0	0	0	0	0
		4	4	1	0	0	0	1
	N	0	0	0	0	0	0	0
		3	0	0	0	0	0	0
15 min. . . .	AH	1	1	2	2	4	4	4
		4	4	4	4	4	4	4
	CAV	0	0	0	0	0	0	0
		1	0	0	0	0	0	1
	N	0	0	0	0	0	0	0
		1	0	0	0	0	0	0
30 min. . . .	AH	0	0	1	2	3	3	3
		1	3	4	4	4	4	4
	CAV	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
60 min. . . .	AH	0	0	0	0	2	3	3
		1	1	3	4	4	4	4
	CAV	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
		0	0	0	0	0	0	0

AH = Acute hepatitis.

N = Normal.

* Readings after 20 hours incubation at 5° C.

† Readings after 40 hours incubation at 5° C.

CAV = Carcinoma ampulla of vater.

Influence of Preliminary Inactivation of Serum at 56° C. on the Titration Test. The effect of preliminary inactivation of serum at 56° C. upon the serial dilution test incubated at 5° C. is shown in Table 4. Heating for as little as 15 minutes reduced the intensity of the flocculation in the fore-zone of tests read after 20 hours incubation and heating for 60 minutes abolished it completely in both normal and pathologic sera. When the serum-lipid mixtures were permitted to stand for 40 hours at

were obtained with all antigens. The lipid emulsions, A, B and E, were most sensitive whereas C and D yielded weaker flocculation with the same sera. These data indicate that standardization of stock etherized cephalin cholesterol preparations is of prime importance in order to obtain comparable results. Such a procedure is now possible with the modified antigen emulsion which permits utilization of serum for long periods of time. Stored normal and abnormal sera with known

patients, patients with the most pronounced jaundice had the lowest values. In the majority of the cases, the values were in the range of 1.0 to 2.0. The values were not significantly different from the values of the normal subjects.

The results of the tests for the presence of H₂ and H₃ antigens in the serum of the patients with jaundice were also determined. The results of the tests for the presence of H₂ and H₃ antigens in the serum of the patients with jaundice were also determined. The results of the tests for the presence of H₂ and H₃ antigens in the serum of the patients with jaundice were also determined.

The results of the tests for the presence of H₂ and H₃ antigens in the serum of the patients with jaundice were also determined. The results of the tests for the presence of H₂ and H₃ antigens in the serum of the patients with jaundice were also determined. The results of the tests for the presence of H₂ and H₃ antigens in the serum of the patients with jaundice were also determined.

TABLE 6. Results of the tests for the presence of H₂ and H₃ antigens in the serum of the patients with jaundice.

No. of patients	H ₂ antigen		H ₃ antigen					
	24 hr.	48 hr.	1	2	3	4	5	6
Standard Tests With the Hanger Antigen "H"	8	8	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
Standard Tests With the Modified Antigen "M"	8	8	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
Standard Tests With the Hanger Antigen "H"	8	8	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
Standard Tests With the Modified Antigen "M"	8	8	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
Standard Tests With the Hanger Antigen "H"	8	8	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
Standard Tests With the Modified Antigen "M"	8	8	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1

Note: Results of the tests for the presence of H₂ and H₃ antigens.

tion data are presented under 3 headings in Table 6: (1) Standard Tests With the Hanger Antigen "H"; the readings are recorded for 24 hour* periods but 48 hour readings, not included in the table, showed no significant changes. (2) Standard Tests With the Modified Antigen "M"—which were read after 48 hours. The difference between 24 and 48 hour readings were, in

of Table 6 utilizing 1 mg. per 100 ml. as the upper limit of normal. Four of the subjects developed clinical jaundice; the remaining patients showed suggestive findings such as anorexia, nausea, vomiting, right upper quadrant tenderness or liver enlargement but laboratory evidence of hepatic disease was not demonstrated. A study of the table reveals that the cephalin

* The 24 hour reading is preferred by some investigators.

cholesterol flocculation test with the Hangar antigen yielded distinctly positive results with the onset of jaundice. Similarly the new antigen as utilized in the standard test proved to be of approximately the same order of sensitivity except in Case 4 where it caused flocculation during convalescence which was not manifest by the Hanger preparation. The titration tests seemed to follow the course of the hepatitis with greater accuracy and showed progressive changes in the degree of flocculation during the transi-

tion was at least as sensitive as the standard tests and more readily interpreted. All 3 procedures yielded negative or doubtful results during convalescence even in the presence of clinical jaundice, a finding noted previously by Hangar.⁵

Results of Titration Tests. Summary data showing the cumulative score of normal and abnormal sera are presented in Table 7. For purposes of simplification the sera have been grouped into those with a score of 5 or less, 6 to 10, 11 to 15, and over 15. Among 100 ambulatory

TABLE 6.—STANDARD AND TITRATION FLOCCULATION TESTS IN INFECTIOUS HEPATITIS

Case	Antigen	Days from inoculation														
		0	10	21	31	42	46	52	56	63	73	84	94	105	115	
1 . . .	H	0	0	0	0	4										
	M	0	0	0	0	3										
	T	0	0	0	0	24										
2 . . .	H	0	0	0	0	3	3	4	4	0	0	0	0	0	0	
	M	1	0	0	0	2	2	3	0	0	0	0	0	2	0	
	T	2	0	1	6	25	21	23	9	0	0	0	1	0	5	
3 . . .	H	0	0	0	0	2	3	3	4	0	0	0	0	0	0	
	M	0	0	0	0	0	3	4	3	1	0	0	0	0	0	
	T	0	0	0	2	21	28	20	10	1	0	0	0	0	0	
4 . . .	H	0	2	1	0	2	4	4	4	4	0	0	0	0	0	
	M	0	0	1	0	2	2	3	4	3	1	3	0	3	0	
	T	1	3	5	7	13	18	28	27	19	2	8	7	10	3	
5 . . .	H	0	0	0	0	0	0	0	3	0	0	0	0	0	0	
	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	T	2	0	0	1	3	2	0	1	4	2	2	4	6	0	
6 . . .	H	0	0	0	0	0	0	0	2	0	0	0	0	0	0	
	M	0	0	0	0	0	0	2	0	0	0	0	0	0	0	
	T	2	0	1	1	2	0	0	2	0	1	1	3	7	0	
7 . . .	H	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
	M	0	0	0	0	1	0	0	0	0	0	0	0	0	0	
	T	3	..	4	4	6	2	4	1	1	3	5	4	5	8	
8 . . .	H	0	1	0	0	2	2	0	1	0	1	0	0	2	3	
	M	1	0	0	0	0	1	2	0	0	1	0	2	1	0	
	T	1	1	3	2	1	3	1	0	2	2	6	1	6	2	

H = Hanger antigen; 24 hour reading expressed 0 to 4+.
M = Modified antigen; 48 hour reading expressed 0 to 4+.
T = Titration; 20 hour reading at 5° C. expressed as cumulative score from 7 tube test with an initial serum dilution of 1 to 4.
The italic figures represents the period during which the blood bilirubin exceeded 1 mg. per 100 ml.

tion from acute illness to convalescence. Furthermore, on the 42nd day following inoculation the titration procedure was positive in Cases 3 and 4 prior to the onset of clinical jaundice while the standard tests showed equivocal results in the form of 2+ reactions which also appeared at times with the sera from patients who did not develop hepatitis (Cases 6 and 8). Although more frequent samplings in the pre-icteric phase would have been advantageous in establishing this point, the data suggested that the titration procedure

adult persons in apparently normal health 95 scored 5 or less in the titration test. The remaining 5% ranged between 6 and 10. A second group of 100 sera were obtained from patients in hospital who were suffering from illnesses other than liver disease. Of these individuals, 98% scored 10 or less in the flocculation titration test. From the 2 persons with scores exceeding 15, second specimens of serum were obtained and both scored 8 on retesting. The term "infected but not ill" refers to 11 subjects who received potentially ictero-

genic material but did not develop hepatitis. Serum was obtained at approximately 10 day intervals for a period of 5 months and finally examined by the serial dilution method. All 68 specimens were scored as 10 or less with the procedure used. The above 3 groups of individuals were utilized for the purpose of establishing the range and degree of flocculation which could be expected in normal persons and in patients with diseases other than those involving the liver. From these data it was concluded that a score of 0 to 5 with a lipid emulsion of known sensitivity was normal, scores of 6 to 10 were classed as doubtful and worthy of further study.

a particular serum to flocculate the lipid emulsion. Grouping of convalescent hepatitis sera in relation to the day of illness revealed that after 15 to 20 days, flocculation scores exceeding 11 were obtained in 3 sera. Sera drawn from 31 to 40 days after the onset yielded positive reactions in 22 sera and samples 40 days after illness scored less than 5 in all instances. These findings are in agreement with those obtained with the usual Hanger procedure in that flocculation tended to decrease as convalescence progressed.

Discussion. Attempts to utilize a dilution method in the cephalin cholester flocculation test have been previously reported (1). In 1943 Bauer¹ suggested that

TABLE 7. SUMMARY DATA OF TITRATION TESTS WITH NORMAL AND ACUTE HEPATITIS SERA

Type of Serum	Normal		Acute Hepatitis		Convalescent Hepatitis		Total	
	No.	Score	No.	Score	No.	Score	No.	Score
Normal	10	10	10	10	10	10	30	30
Acute Hepatitis	10	10	10	10	10	10	30	30
Convalescent Hepatitis	10	10	10	10	10	10	30	30
Total	30	30	30	30	30	30	90	90

A total of 45 sera from 35 patients with infectious hepatitis of 14 or less days duration was examined by the titration procedure (Table 7). Five samples reacted with a score of 11 to 15 and were, therefore, classed as weakly positive. Strong flocculation was exhibited by 37 sera each with cumulative scores exceeding 15; 3 specimens, none of which appeared grossly icteric, scored 5 or less; 2 of these were obtained from 1 patient on the 10th and 14th days of illness; the other was drawn on the 5th day of disease. From the above data it was concluded that the titration method was sufficiently sensitive to detect more than 90% of infectious hepatitis in the acute phase.

The sera from convalescent hepatitis, cirrhosis and tumors involving the liver parenchyma showed intermediate types of reactions which varied with the ability of

tenfold dilutions of serum were more useful for the interpretation of results than the single tube test. This contention, however, was handicapped by the lack of normal serum controls as pointed out shortly thereafter by Mirsky and von Brecht.¹² They found that 0.2 ml. of serum in the standard test gave satisfactory negative results but that reduction in practically all normal specimens. For this reason they did not consider the Bruger procedure to be a valid one for the detection of alterations in hepatic function. We have obtained similar results with the Hanger type of antigen in titration tests carried out at 5° C. Such false positive reactions with normal sera may perhaps be due to dilution of the inhibiting albumin component as suggested by the data of Moore *et al.*¹³ in their studies of the mechanism

responsible for the cephalin-cholesterol flocculation. Modification of the method of preparation of the antigen-emulsion has resulted in a reduction of flocculation in all zones of dilution of normal serum, thus permitting the use of a titration technique. A study of flocculation over a range of serum dilutions has the additional advantage of minimizing errors frequently associated with single tube tests.

With the modified antigen it is no longer necessary to limit tests to freshly drawn sera. Specimens may be stored and examined under the same conditions with a single lot of standardized cephalin cholesterol emulsion. Furthermore, in equivocal cases the sera may be reexamined for comparison with sera obtained later in the clinical course. The elimination of interfering photosensitivity and error due to prolonged standing of serum in the diluted form represents further advantages of the new procedure. The selection of 5° C. as the temperature for incubation was not entirely based on the evidence presented in Table 3. In experiments not shown in the protocols certain acute hepatic sera flocculated the antigen poorly at 21° C. but strongly at 5° C. Other factors which influenced the decision to use ice-box incubation were the variability in room temperature throughout the year and the possibility of bacterial contamination. The data suggest a number of factors in the flocculation test which remain to be studied. The variation among different commercial lots of antigen as seen in Table 5 point to the necessity of introducing proper standardization procedures. The selective elimination of flocculation with normal sera by heating to 56° C. appears possible from the evidence in Table 4.

Some acute sera from patients in whom

a diagnosis of infectious hepatitis had been made failed to react at all, a finding which did not agree with the clinical impression. Negative reactions under these conditions may either indicate an error in diagnosis or mild parenchymatous disease. In the latter instances and during convalescence the fluctuations noted in the titration test may have greater prognostic significance than the limited variations which can be recorded with the standard Hanger procedure.^{2,3,5,6,9,14,16,17} This opinion is illustrated by the data presented in Table 6 where the sera of Subjects 2 and 3 showed a sharp decline in cumulative score while the Hanger test remained 4+; with Patient 4, the prolonged illness was accompanied by a continued abnormality in the cumulative score, after the Hanger test had become negative.

Summary. 1. Confirmation is reported of observations by others that cephalin cholesterol emulsions prepared by the Hanger technique are flocculated by normal sera which have been diluted and exposed to light or permitted to age.

2. A modified method of preparing a cephalin cholesterol emulsion from the stock etherized solution is described and some factors influencing the sensitivity of this antigen are presented.

3. The modified emulsion is not flocculated with normal sera which have been exposed to light, diluted, or aged. It does, however, flocculate satisfactorily in the presence of sera from patients with parenchymatous liver diseases.

4. Tests made with serial dilutions of serum and utilizing the modified emulsion permits more accurate interpretation of the flocculation pattern in liver disease than does the procedure of Hanger.

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THE EFFECT OF PROSTIGMINE (NEOSTIGMINE) ON THE MUSCLE SPASM OF RHEUMATOID ARTHRITIS*

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PROSTIGMINE has been advocated in recent years for the relief of muscle spasm in anterior poliomyelitis and in a variety of diseased states of the central nervous system.^{2,3,5,4,9,13} More recent work has suggested that prostigmine might be of value for the relief of muscle spasm associated with arthritic conditions.^{8,17} The purpose of the present study was to evaluate the effect of prostigmine on the muscle spasm of rheumatoid arthritis.

Prostigmine (neostigmine) is a synthetic alkaloid belonging to the physostigmine group of drugs. Its fundamental pharmacologic action is thought to be due primarily to its inactivation of the enzyme cholinesterase, thus diminishing the breakdown of acetylcholine throughout the body. Acetylcholine is the chemical mediator of nerve impulses to the end-organs of the voluntary nervous system and of the cholinergic division of the autonomic nervous system.¹ Atropine can block the cholinergic effects of prostigmine on the vagal end-organs and the sweat glands, but does not have this effect on the myoneural junction of skeletal muscle.⁷ Under the influence of toxic amounts of prostigmine, fasciculations of skeletal muscle occur in spite of atropinization. Thus it appears that prostigmine lowers the threshold of response of skeletal muscle, making it more irritable by preserving

acetylcholine at the myoneural junctions. and to this is attributed its well-known therapeutic value in myasthenia gravis. The action of prostigmine on the central nervous system is less well understood. If, as postulated by Loewi,¹¹ acetylcholine acts as the chemical transmitter of nerve impulses in the synapses of the brain and spinal cord, prostigmine might well be expected to be an excitant of the central nervous system and to increase the ease of transmission of nerve impulses through the central nervous system. However, to the contrary, most observers^{10,12,14,15,16} have found that prostigmine administered either intrathecally or intravenously depresses the spinal reflexes in mammals and man. The physiologic mechanism by which this depression of the spinal reflex arc is brought about is not clearly understood. However, it has been shown that while small doses of acetylcholine stimulate nerve synapses, larger doses paralyze them.¹¹ It is probable that the action of prostigmine on the central nervous system is due to its acetylcholine sparing effect. Atropine does not block the action of prostigmine on the spinal cord.¹⁵

Prostigmine was first suggested for use in combating muscle spasm in anterior poliomyelitis by Kabat and Knapp⁹ in 1943. They treated 20 cases of subacute poliomyelitis with a combination of sub-

* Read before the meeting of the American Rheumatism Association, May 27, 1946.

TABLE 1.—EFFECTS OF PROSTIGMINE THERAPY IN CASES OF RUPR MAXIMO ANGINUS

Case	Involved joints	Pre-treatment					Post-treatment				
		Motion before	Dysmetria	Effects	Dysmetria	Post-treatment	Dysmetria	Post-treatment	Post-treatment	Post-treatment	Post-treatment
1	L. knee	110°	4	111°	Relaxed, normal	3	110°	110°	110°	110°	110°
2	R. shoulder—abduction	110°	5	120°	Shoulder relaxed	5	115°	Shoulder relaxed	115°	Shoulder relaxed	115°
	R. elbow	90°	5	105°	Relaxed, normal	5	110°	Relaxed, normal	110°	Relaxed, normal	110°
	R. wrist—flex.—ext.	70°	5	70°	Relaxed, normal	5	110°	Relaxed, normal	110°	Relaxed, normal	110°
	R. hip—flex.—ext.	100°	5	110°	Relaxed, normal	5	125°	Relaxed, normal	125°	Relaxed, normal	125°
	L. hip—flex.—ext.	100°	5	125°	Relaxed, normal	5	125°	Relaxed, normal	125°	Relaxed, normal	125°
3	L. knee	90°	5	95°	Shoulder relaxed	5	95°	Shoulder relaxed	95°	Shoulder relaxed	95°
	L. knee	75°	5	85°	Shoulder relaxed	5	85°	Shoulder relaxed	85°	Shoulder relaxed	85°
	L. elbow	90°	5	95°	Shoulder relaxed	5	95°	Shoulder relaxed	95°	Shoulder relaxed	95°
4	R. knee	115°	3	115°	Relaxed, normal	3	115°	Relaxed, normal	115°	Relaxed, normal	115°
	L. knee	115°	3	115°	Relaxed, normal	3	115°	Relaxed, normal	115°	Relaxed, normal	115°
5	R. elbow	90°	3	95°	Shoulder relaxed	3	95°	Shoulder relaxed	95°	Shoulder relaxed	95°
	R. wrist—flex.—ext.	50°	3	55°	Shoulder relaxed	3	55°	Shoulder relaxed	55°	Shoulder relaxed	55°
	R. wrist—flex.—ext.	50°	3	55°	Shoulder relaxed	3	55°	Shoulder relaxed	55°	Shoulder relaxed	55°
	L. wrist—flex.—ext.	50°	3	55°	Shoulder relaxed	3	55°	Shoulder relaxed	55°	Shoulder relaxed	55°
6	R. knee	100°	3	110°	Shoulder relaxed	3	110°	Shoulder relaxed	110°	Shoulder relaxed	110°
	L. shoulder—abduction	90°	3	95°	Shoulder relaxed	3	95°	Shoulder relaxed	95°	Shoulder relaxed	95°
	R. wrist—flex.—ext.	120°	3	125°	Shoulder relaxed	3	125°	Shoulder relaxed	125°	Shoulder relaxed	125°
	L. wrist—flex.—ext.	120°	3	125°	Shoulder relaxed	3	125°	Shoulder relaxed	125°	Shoulder relaxed	125°
8	L. hip—flex.—ext.	100°	6	105°	Shoulder relaxed	6	105°	Shoulder relaxed	105°	Shoulder relaxed	105°
	R. hip—flex.—ext.	100°	6	105°	Shoulder relaxed	6	105°	Shoulder relaxed	105°	Shoulder relaxed	105°
9	Spine—flexion: fingers to floor	90°	1	95°	Shoulder relaxed	1	95°	Shoulder relaxed	95°	Shoulder relaxed	95°
	S.L.R.—R.	100°	1	105°	Shoulder relaxed	1	105°	Shoulder relaxed	105°	Shoulder relaxed	105°
10	Spine—flexion: fingers to floor	120°	3	125°	Shoulder relaxed	3	125°	Shoulder relaxed	125°	Shoulder relaxed	125°
	S.L.R.—R.	120°	3	125°	Shoulder relaxed	3	125°	Shoulder relaxed	125°	Shoulder relaxed	125°
12	Spine—flexion: fingers to floor	120°	3	125°	Shoulder relaxed	3	125°	Shoulder relaxed	125°	Shoulder relaxed	125°
	S.L.R.—R.	120°	3	125°	Shoulder relaxed	3	125°	Shoulder relaxed	125°	Shoulder relaxed	125°
13	Spine—flexion: fingers to floor	120°	3	125°	Shoulder relaxed	3	125°	Shoulder relaxed	125°	Shoulder relaxed	125°
	S.L.R.—R.	120°	3	125°	Shoulder relaxed	3	125°	Shoulder relaxed	125°	Shoulder relaxed	125°

TABLE 2. EFFECT OF A SINGLE DOSE OF PROSTIGMINE BROMIDE ON SPASM OF RHEUMATOID ARTHRITIS
45 mg. prostigmine bromide and 0.6 mg. atropine sulfate orally

Case	Involved joints	1 hour after dose						2 hours after dose						4 hours after dose						Benefit
		Motion before treatment			Effects			Motion			Effects			Motion			Effects			
		Motion	Range	Quality	Sl. vertigo; no relaxation	Sl. vertigo; no relaxation	Sl. vertigo; no relaxation	Motion	Range	Quality	Motion	Range	Quality	Motion	Range	Quality	Motion	Range	Quality	
17	L. shoulder—abduction flexion R. shoulder—abduction flexion	60° 30° 60° 40°	3 3 3 3	55° 35° 55° 35°	7 7 7 7	Sl. vertigo; no relaxation	7 7 7 7	None None None None	55° 40° 50° 40°	7 7 7 7	60° 40° 55° 40°	7 7 7 7	None None None None	60° 40° 55° 40°	7 7 7 7	None None None None	60° 40° 55° 40°	7 7 7 7	None None None None	None + 10° — 5° None
18	R. elbow	115°	3	110°	2	No effect	2	Muscle twitching	115°	2	110°	2	Muscle twitching	110°	2	Muscle twitching	110°	2	Muscle twitching	— 5°
19	R. hip—flexion abduction R. knee	55° 50° 120°	3 3 3	60° 50° 120°	2 2 2	No effect; extra 0.6 mg. atropine given	2 2 2	No effect	60° 40° 120°	2 2 2	60° 40° 120°	2 2 2	Abdominal cramps mod.	60° 40° 120°	2 2 2	Abdominal cramps mod.	60° 40° 120°	2 2 2	Abdominal cramps mod.	+ 5° — 10° None
20	L. ankle—invers.—evers. flex.—ext.	10° 40°	3 3	10° 45°	2 2	No effect	2 2	Mild muscle twitching	10° 40°	2 2	10° 40°	2 2	No effect	10° 40°	2 2	No effect	10° 40°	2 2	No effect	None
21	L. elbow L. knee R. knee	70° 125° 95°	3 3 3	70° 120° 95°	2 2 2	Sweating; reflexes more active	2 2 2	Deer. sweating; more relaxed	70° 125° 97°	2 2 2	70° 125° 95°	2 2 2	Abdominal cramps mod.	70° 125° 95°	2 2 2	Abdominal cramps mod.	70° 125° 95°	2 2 2	Abdominal cramps mod.	None None None
22	L. knee	90°	3	85°	2	Severe twitch.; more atropine given, 1 mg.	2	Severe twitch., cramps and nausea	85°	2	90°	2	Persistent twitch.; reflexes increased	90°	2	Persistent twitch.; reflexes increased	90°	2	Persistent twitch.; reflexes increased	None
23	L. wrist—flex.—ext. R. wrist—flex.—ext. Neck—rotation flex.—ext. Int. deviation	20° 30° 60° 70° 40°	3 3 3 3 3	20° 30° 60° 70° 30°	2 2 2 2 2	Reflexes more active; given atropine, 0.6 mg.	2 2 2 2 2	Abdominal cramps and muscle twitching	20° 30° 50° 60° 50°	2 2 2 2 2	20° 30° 50° 60° 45°	2 2 2 2 2	Occas. muscle twitching	20° 30° 60° 65° 45°	2 2 2 2 2	Occas. muscle twitching	20° 30° 60° 65° 45°	2 2 2 2 2	Occas. muscle twitching	None None None + 5° + 5°

cutaneous and oral prostigmine and noted in all cases a significantly increased range of motion of the afflicted limbs and in some instances a decrease in deformities by relaxation of muscle hypertonus. They theorized that the beneficial response obtained was due to the anticholinesterase effect of prostigmine on the synapses of the internuncial neurons. The neurons they take to be functionally impaired in poliomyelitis. Other investigators^{11,12} have made similar clinical observations with prostigmine in acute and subacute anterior poliomyelitis, though in their cases the relaxation of muscle spasticity was much less dramatic than in the series of Kubat and Knapp.⁹ Further studies in anterior poliomyelitis were carried out by Watkins and Brazier¹³ who quantitatively measured the electrical discharges from the spastic muscles before and after the intramuscular injection of prostigmine. They were unable to detect changes after use of prostigmine which they could accept as of significant degree to indicate a specific effect on the muscle spasm.

Trommer and Cohen¹⁴ applied the principles of Kubat and Knapp's work in poliomyelitis to various joint diseases with associated muscle spasm. They reported that, in 13 of their 19 cases treated with prostigmine (including some with rheumatoid arthritis), the muscle spasm was diminished. Kubat⁹ has also reported that prostigmine reduced muscle spasm in patients having various types of joint and muscle disease. Thus prostigmine has been put forth by these authors as beneficial in releasing the muscle spasm of rheumatoid arthritis and allied conditions.

Method. Prostigmine was administered to 23 patients with active rheumatoid arthritis. All patients were hospitalized during the period of prostigmine therapy and under the constant surveillance of the authors. Of the cases, 13 were rheumatoid arthritis with various peripheral joints involved, 8 cases were rheumatoid spondylitis with involvement of the sacroiliac joints and spine, and 2 cases had involvement of both the spine and sacro-

ilic joint as well as peripheral joint. All cases were relatively early in the course of their present attack. Activity in each instance was manifested subjectively by malaise, pain and stiffness of the affected joint, and objectively by weight loss, swollen tender joints with spasm of the surrounding muscles, and elevated erythrocyte sedimentation rates. The patients with spondylitis had Roentgen ray abnormalities in the sacroiliac joints, decreased chest expansion, limitation of straight leg raising (S.L.R.), marked spasm of the erector spinae muscles, and limitation of motion of all or part of the spine. None of the cases had ankylosed joints or advanced contracture. A goniometer was employed to determine in degree the maximum range of motion of the affected joints before prostigmine was administered and at various subsequent intervals. In patients having spondylitis, goniometric determinations were made of the angle of straight leg raising (S.L.R.). Flexion of the spine was estimated by measuring the distance from the finger-tips to the floor with the patient flexing the spine with the knees in extension. All patients had been receiving physical therapy for varying periods and this was continued uninterrupted during the present study.

In each of the first 16 cases studied (Table 1) 0.5 mg. prostigmine methyl sulfate and 0.6 mg. atropine sulfate were injected subcutaneously. Three hours after the initial dose, prostigmine bromide was administered orally in doses, which, if tolerated, increased from 15 to 30 to 45 mg. 3 times each day. Atropine sulfate (0.6 mg.) was given with each dose of prostigmine. Determinations of joint mobility were made prior to each increase in the dose of prostigmine and on terminating therapy.

Seven cases were later studied (Table 2) to determine the immediate effect of a single large dose of prostigmine on muscle spasm. Each of these patients was given 45 mg. of prostigmine and 0.6 mg. atropine sulfate orally. Observations including

measurements of range of joint motion were made at 1, 2 and 4 hours after administration.

Discussion. As shown in Table 1, only 2 cases showed an increase in range of motion of more than 10° following the use of prostigmine. Our experience and that of others⁵ is that goniometric measurements are reliable only to within 10° . Because of minor errors in measurements and the known daily variation in intensity of muscle spasm in rheumatoid arthritis, we think that changes of 10° or less are not significant. Of the 2 cases which did show more than 10° increased motion in some of the affected joints, 1 (Case 5) showed a concomitant decreased range of motion of 15° in 1 of the 3 joints studied. The other case (Case 2) showed a significant increased range of motion in all 6 joints measured. However, it later became evident that this patient's arthritis was going into a spontaneous remission at the time these studies were undertaken. Some degree of subjective improvement was noted by 6 patients (Cases 1, 2, 3, 4, 6, 14) at some time during the study, but in only 1 of these (Case 2) was there significant objective evidence of a decrease in muscle spasm.

Three cases could not tolerate more than 15 mg. of prostigmine 3 times a day in spite of atropinization. Seven cases could take only 30 mg. 3 times a day, and 6 cases 45 mg. 3 times a day. There was no correlation between the amount of drug tolerated and the degree of benefit noted.

None of the 7 patients given a single massive dose of prostigmine showed any significant objective release of muscle spasm as manifested by increased range of motion in the affected joints, and none experienced any subjective improvement. In fact, 6 of the 7 cases developed unpleasant toxic reactions. Four showed generalized muscular fasciculations and 2 had abdominal cramps throughout the period of observation.

The disparity between our findings with prostigmine in rheumatoid arthritis and

those of other observers in anterior poliomyelitis possibly may be attributed to the different origin of the muscle spasm in the 2 conditions. In anterior poliomyelitis the muscle spasm is a complex phenomenon due to inflammation in the spinal cord with secondary changes in the muscles themselves. In rheumatoid arthritis, on the other hand, the spasm is due to prolonged guarding against movement of a painful joint, and also to inflammatory changes in the muscles, fibrous tissue and peripheral nerves as described by Freund, Steiner, Lichtentritt and Price.^{5,6} The central action of prostigmine, such as it may be, is apparently insufficient to affect significantly the muscle spasm of rheumatoid arthritis. Previous observations have confirmed that the action of prostigmine on skeletal muscle is that of an excitant, and this explains the muscular fasciculations seen in so many of our cases. Although this theory might explain the failure of prostigmine to control spasticity in rheumatoid arthritis, even though effective in diseases such as poliomyelitis, we cannot explain the obvious difference between our results and those reported by Trommer and Cohen,¹⁷ and Kabat⁸ in rheumatic conditions.

From our data we conclude that prostigmine induces no significant relaxation of muscle spasm in rheumatoid arthritis. The slight variation in range of motion noted in our cases can be accounted for by the daily variations caused by changes in weather, fatigue, or other influences which so commonly make the rheumatic patient better on some days and worse on others. We have avoided emphasis on subjective benefit, as many rheumatic sufferers are highly suggestible, often expressing some relief from placebos alone. Objective findings are little influenced by the receptiveness of the individual to a new form of therapy, and so are more reliable. In our opinion, a potent drug such as prostigmine should yield definite benefit to justify its continued use in rheumatoid arthritis. This we have not found.

Summary. 1. Prostigmine in doses up to the limit of tolerance was administered to 23 patients with rheumatoid arthritis in an attempt to relieve the associated muscle spasm.

2. In no case was a satisfactory therapeutic response obtained. Only 2 cases showed any appreciable decrease in muscle spasm, and even in 1 of these the

effect was inconsistent, as the spasm about other affected joints coincidentally increased. The second case had already started into spontaneous remission.

3. Prostigmine caused unpleasant and often alarming reactions in 14 of the 23 cases studied.

4. We do not recommend prostigmine in the treatment of rheumatoid arthritis.

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MELITURIA IN HEALTHY AMERICAN MEN WITH SPECIAL REFERENCE TO TRANSITORY GLYCOSURIA

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WHENEVER any large group of individuals presumably healthy are surveyed by urine analysis, a certain small percentage will be found to be excreting reducing substances at the hour of the survey. Opportunities to discover and study such questionably abnormal individuals have become more numerous in the past few years, as a direct consequence of the fitness examinations being given to millions of young men and women prior to their entry into the fighting forces. The present paper summarizes the experiences and follow-up studies made on 77,293 consecutive male enrollees received at a large Maritime Service Training Station. When screened by urine analysis within the 1st day or 2 of reporting for duty, 324 (0.42 %) gave a positive reaction to the Benedict test for sugar in the urine.

Few reports on the incidence of melituria in recruits for this war have as yet appeared. Peel and Peel⁹ studied some 115 cases of glycosuria encountered in an unspecified number of men called up for service with the British Army. Of these, 43 were classed as clear-cut diabetes, 42 as borderline diabetes, and 30 as renal glycosuria. Blotner and Hyde² recounted their experience with 45,650 consecutive military inductees from the Boston area. Melituria was found in 367 (0.8 %), divided as follows: diabetes mellitus, 208 (0.46 %); transient glycosuria, 126 (0.28 %) renal glycosuria, 3 (0.07 %). Spellberg and Leff¹¹ reported on 32,033 similar inductees seen at New Orleans; 37 instances of glycosuria were found, of whom only 9 were diagnosed as having diabetes mellitus. The etiology of the sugar in the urine of the non-diabetics was not investigated. None of these reports gives information as to whether the population of eligible

males from which the inductees were drawn had been screened by prior physical examination including urine analysis.^{13b}

During the war years the number of men volunteering for Maritime Service training was always greater than the need. It was the custom, therefore, to reject summarily, at the enrolling offices, all applicants found with sugar in the urine. Further studies were made only when evidence was submitted from outside sources that the melituria was benign, or when an enrolling physician chanced to take a special interest in some applicant. The incidence of the varieties of melituria in the series here reported is therefore not representative for the population as a whole. The findings portray conditions existent in a selected group of American adult males, adjudged to be in good health by a physical examination received within the preceding few weeks.

For strict accuracy no individual with melituria should be diagnosed as having glycosuria or dextrosuria until the excreted sugar has been actually identified as dextrose. Such identification can be carried out with reasonable reliability by any clinical laboratory, though incontrovertible recognition requires skill, time, and much special equipment. Dextrose and also levulose become completely destroyed on incubation with baker's yeast at 30° C. for 2 to 6 hours, whereas galactose and pentose resist fermentation under these circumstances. Levulosuria (fructosuria) is so rare that any yeast-fermentable reducing substance found in urine may be held to be dextrose without further investigation, unless the attendant circumstances are peculiar.

"Pseudomelituria" may be met with at times, due to the presence in the urine

of reducing substance other than sugar. The best way to avoid such false reactions is by correct application of the test procedures. When properly carried out in the recommended proportions Benedict's test is rather highly specific.¹² With no more than 2 minutes boiling over a flame, or 3 minutes in a water-bath, this reagent has a threshold sensitivity of about 50 mg. of sugar per 100 ml. of urine (0.05%). The false positives it gives are usually traceable to more prolonged exposure to heat. It is much less prone than tests of the Fehling or Trommer type to give positive reactions in the absence of a true sugar.

Classification and Criteria. 1. **TRANSITORY MELITURIA.** (a) *Alimentary.* Fruits or fruit juices in excess evoke saccharuria or glucosuria; candy or toast may induce dextrosuria, maltosuria, or dextrinuria; excess lactose or galactose results in lactosuria or galactosuria. Overflow meliturias of this sort, of dietary origin, are transitory and harmless. Of these sugars the only ones which will ordinarily be picked up by Benedict's test are maltose and dextrose.

(b) *Toxic.* Patients ill with sundry disorders sometimes excrete dextrose in the urine. This has been reported in pregnancy, furunculosis, lead poisoning, cerebral conditions, hyperthyroidism, hypopituitarism, and febrile states.

(c) *Emotional.* High emotional strain can excite a temporary dextrosuria. The current physiologic explanation of this phenomenon, based on Cannon's cat experiments, is that under strong emotion, such as fright, epinephrin becomes secreted in augmented degree.^{4,6} The epinephrin mobilizes hepatic glycogen and transitorily raises the blood sugar level above the renal threshold.

2. **RENAL GLYCOSURIA.** This condition is often familial. The excretion of dextrose is chronic, continuous, non-progressive, and harmless. The quantity of dextrose excreted differs from patient to patient; it may fluctuate with variations in carbohydrate intake but in the more

marked instances is largely independent of diet. Reduction of blood sugar by insulin may transiently inhibit the excretion. In accord with Marble's⁷ recommendation, only those individuals who excrete dextrose continuously through day and night should be diagnosed as having renal glycosuria.

3. **LOW RENAL THRESHOLD.** This diagnosis is reserved for those non-diabetic individuals who have negative urine when fasting but who excrete sugar following a meal containing carbohydrate. Mosenthal and Ashe⁸ have described how the blood threshold level for dextrose excretion may range between 100 and 160 mg. per 100 ml. The latter figure is commonly taken as the lower limit of normal renal threshold for dextrose.

In both renal glycosuria and low renal threshold there are no symptoms and no hyperglycemia, either during fasting or after a meal. The dextrose tolerance test reveals a normal or unusually flat curve. Treatment is not needed. Every newly discovered case should be observed medically for some years, inasmuch as rare patients with beginning diabetes mellitus may simulate these conditions.

4. **DIABETES MELLITUS.** The diagnostic tool relied upon most for establishing the presence or absence of diabetes is the dextrose tolerance test, in one or another of its variant forms. To ascertain the approximate renal threshold urine samples are tested at the same times that blood specimens are taken. With the 1 dose-3 hour test, the fasting blood sugar is first determined, 100 gm. of dextrose are given orally, and specimens of venous blood collected at $\frac{1}{2}$, 1, 2 and 3 hour intervals. The standards taken for top normal response are: a fasting reading of less than about 0.12 gm. sugar per 100 ml. of blood, a $\frac{1}{2}$ hour value of less than about 0.18 gm. per 100 ml., and a return to 0.12 mg. by 2 hours. With the Exton-Rose 2 dose-1 hour test the fasting blood sugar is first determined, 50 gm. of dextrose are given orally, a second blood specimen is taken after 30 minutes, a

second dose of 50 gm. dextrose given, and a final blood specimen collected 30 minutes later. The standards taken for top normal response are: a fasting level of less than about 0.12 gm. per 100 ml., and $\frac{1}{2}$ and 1 hour blood sugar values no higher than 0.16 to 0.18 gm.⁵ Prior to performing either of these tests—and borderline cases receive both—300 gm. of dextrose solution in excess of the normal diet is usually fed for 2 or 3 days, in order to stimulate the insulin-producing mechanisms. Thus are avoided the confusing results so often obtained when the antecedent diet has been low in carbohydrates or inadequate in calories.

Some physicians are content to exclude the diagnosis of diabetes mellitus in suspects whose arrival specimens contained sugar, if additional specimens, collected postprandially, are sugar-free. Without doubt this practice rules out well-established diabetes (if untreated) but mild or incipient cases can slip through a screen consisting solely of repeated urine tests. Such incipient cases (the so-called latent or potential or "pre-" diabetics) can be sugar-free after a fast and in the early morning, but will excrete sugar after ingestion of sweets or a large meal. The dextrose tolerance test is a much more sensitive and reliable procedure. In otherwise healthy individuals, under appropriate conditions, "negative reactions to tolerance tests almost always can be accepted with assurance as evidence of absence of diabetes." (Wilder.¹²)

5. **CONGENITAL NON-DEXTROSIC MELITURIA.** Pentosuria, fructosuria, galactosuria and sucrosuria are recognized hereditary abnormalities. Small amounts of the respective sugars are excreted more or less continuously into the urine while the dextrose metabolism remains entirely normal.^{13a} Lactosuria does not occur in adult males.

6. **UNDIAGNOSED.** In this group we have placed all who were transferred to other stations or who disenrolled, voluntarily or otherwise, before diagnostic studies were complete.

Subjects and Methods. Between March 1943 and April 1945, 77,293 consecutive male trainees were admitted to the Sheepshead Bay Training Station of the U. S. Maritime Service. These men were nearly all American citizens, and were recruited in the northeastern quarter of the United States. At the time of reporting they were free from obvious illnesses apart from occasional upper respiratory tract infections or other minor acute disabilities. All had satisfactorily passed physical examinations and presumably had sugar-free urine when enrolled at regional offices in the month prior. Their urine specimens were usually collected for test on the day following arrival at the training station, in mid-morning or mid-afternoon, between 1 and 3 hours after a meal.

The examination period found most of the men fatigued and emotionally upset. Their home ties and business affiliations had just been broken, and a new pattern of living was about to begin. Many had been traveling long distances, on uncomfortable stuffy trains with poor facilities for eating and sleeping. On arrival they had been ushered into an impersonal and bewildering military routine. They were now being taken to the medical department for a physical going-over, with the foreknowledge that painful inoculations would immediately follow.

The urine specimens were tested for sugar with Benedict's qualitative reagent. The tubes were placed in a boiling water bath for 3 minutes exactly, and readings made immediately afterward. Positive specimens were next subjected to Benedict's quantitative test, and the content of reducing substances expressed in terms of dextrose. With every subject having persisting melituria, and with 65 successive individuals having transitory melituria, the sugar in the urine was identified as dextrose in every case by yeast fermentation. It has therefore been assumed that with all subjects in this series the excreted reducing substance has been dextrose.

All men with positive urine findings were called back by the medical department for a clinical history, further urine analyses, and blood studies in most instances.

Results and Discussion. Of the total of 77,293 arriving trainees, 324 (0.42%) exhibited melituria. On diagnostic study

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the great majority of the were found to have melituria of harmless nature. Only 21 (7.7%) of the positive cases had diabetes (Table 1).

The age distribution are given in Table 2 and the breakdown in terms of concentration of sugar in the urine in Table 4. There were no indications with any subject that the excreted sugar was other than dextrose.

known mechanism active in "emotional" glycosuria. Information is not available as to the state of carbohydrate "saturation" of the men at the time of their arrival; but the borderline response were frequently encountered in their first dextrose tolerance tests. The fasting blood sugar level were frequently in the high normal zone, or an unusually high peak appeared in the

Table 1. Diagnostic Breakdown of the 324 Instances of Melituria Found Among 77,291 Testees

Diagnosis	No.	Percent	Percentage
1. Transitory	235	0.29	82.7
2. Low renal threshold	19	0.02	7.0
3. Renal glycosuria	7	0.01	2.6
4. Diabetes mellitus	21	0.01	7.7
5. Undiagnosed (diabetic tendencies, transients, etc.)	52	0.07	100.0
Total	324	0.42	

Table 2. Incidence and Percentage Distribution of Melituria According to Age Groups

Age group (years)	No. testees	Transitory	Low renal threshold	Renal glycosuria	Diabetes mellitus	Undiagnosed	Total
16-20	41,786	88 (0.21%)	9 (0.02%)	4 (0.01%)	0 (0.00%)	15 (0.04%)	125 (0.28%)
21-24	13,781	28 (0.20%)	2 (0.02%)	1 (0.01%)	3 (0.02%)	11 (0.08%)	44 (0.32%)
25-30	10,011	49 (0.49%)	1 (0.01%)	1 (0.01%)	4 (0.04%)	11 (0.10%)	66 (0.65%)
31-34	5,081	15 (0.29%)	4 (0.08%)	2 (0.04%)	1 (0.02%)	8 (0.14%)	29 (0.57%)
35 and over	2,312	21 (0.91%)	3 (0.13%)	1 (0.04%)	4 (0.17%)	7 (0.30%)	36 (1.55%)
Total	77,291	224 (0.29%)	19 (0.02%)	7 (0.01%)	21 (0.03%)	52 (0.07%)	324 (0.42%)

In 82.7% the diagnosis of transitory melituria was arrived at. This large group consisted of men who exhibited reducing substances in the urine but once (or in 31 instances, twice), and in whom neither a diabetically disturbed carbohydrate metabolism nor a permanently lowered renal threshold were demonstrable.

Re-study of this group after a few years, were this possible, might uncover a certain percentage having more advanced disturbances sufficient to admit the recognition of diabetes mellitus.

Many of those whose urines exhibited reducing substances on 2 occasions before becoming consistently negative may have had unrecognized low renal thresholds. Possibly a number of the men had indulged in candy bars or high carbohydrate foods a few hours prior to their voiding of the first specimen. In others the excitement and novelty attendant upon the 1st day of training may have stimulated the un-

first specimen collected after ingestion of the test dose of carbohydrate, or the curve, otherwise normal, failed to drop to the fasting level until 2 hours had passed. On repetition a few days later, after foregoing of carbohydrates, these peculiarities of the blood sugar curve would no longer be found.

The rise in incidence of positive reactions above 25 years of age (Table 2) runs more or less parallel to the familiar age trend for the incidence of diabetes mellitus. This suggests that transitory melituria is an early expression of a lack of flexibility in internal carbohydrate economy. It is of interest that no mention of a possible relationship between transitory melituria and advance in age is to be found in the recent reviews and textbooks consulted as references.

For this group one can say only that at the time and under the circumstances of the screening survey about 1 of every

289 healthy enrollees undergoing examination was found to be temporarily excreting a reducing substance, presumptively dextrose. The possible pathogenesis is discussed further in a later section.

With the 7 cases diagnosed renal glycosuria all urine specimens contained sugar, including those secured when the blood levels were as low as 60 to 80 mg. per 100 ml. Several had positive family histories of the same condition, and 2 were already aware of its existence in themselves. With 4 the dextrose tolerance curves were strikingly "flat." The quantity of sugar excreted by each patient was of a more or less constant order of magnitude in hour-to-hour and day-to-day sampling, the average concentrations being, respectively, 0.5, 0.7, 0.7, 0.8, 1, 1.2, and 1.3 mg. per 100 ml. of urine.

whenever an arbitrary line is drawn, borderline findings are bound to be met. With controversial cases the interpretation and handling fluctuated with the clinical acumen and professional interest of the attending physicians.

Of the 21 patients with diabetes mellitus, 17 were free from symptoms and ignorant of their illness. In 2 men the urine contained ketone bodies as well as dextrose: 1 confessed to having taken insulin for 5 years, the other for 6 years. The 2 others had been rejected recently by the army because of early diabetes. All 4 had hoped to go through the training course without the existence of the diabetes being discovered. Not included in this tabulation are some additional cases of diabetes which came to light during

TABLE 3.—APPROXIMATE DISTRIBUTION OF CRITICAL BLOOD SUGAR VALUES IN 19 CASES HAVING LOW RENAL THRESHOLD (VENOUS BLOOD)

Renal threshold blood sugar (mg. per 100 ml.)	No. cases	Renal threshold blood sugar (mg. per 100 ml.)	No. cases
91-100	2	131-140	3
101-110	2	141-150	6
111-120	0	151-160	4
121-130	2		

In the low renal threshold group exact identification of the urine sugar was not always possible, but non-dextrosic melituria was ruled out by virtue of the fact that these individuals had sugar-free urine when fasting. From the results of the dextrose tolerance tests an effort has been made to estimate the levels of blood sugar at which sugar appeared in the urine. This is not highly exact as a method for determining renal thresholds, but a summary of the observations does give a rough picture of the scattered distribution (Table 3).

The equivocal instances of diabetes mellitus were given the benefit of the doubt. Only those whose carbohydrate metabolism was disturbed beyond question were diagnosed as having diabetes and given medical discharges. Family history was taken into consideration in arriving at the diagnoses. To be sure,

the course of training, men whose urine examinations has been negative on arrival.

No instance of any of the varieties of congenital non-dextrosic melituria were met with among this large series of normal men. This is not surprising since the occurrence rate of pentosuria, which is the most common kind, has been estimated by Blatherwick¹ as 1 in 50,000. Bock³ estimated the prevalence of fructosuria as less than 1 in 100,000.

For the undiagnosed cases the distribution of age and sugar concentration ran closely parallel to those for the total series, indicating the random unbiased character of this group (Tables 2 and 4).

Pathogenesis of Transitory Dextrosuria. Fourteen men with transitory dextrosuria were subjected to more intensive laboratory studies. It was first established by fermentation tests that the reducing sugar they excreted was dextrose.

The hypothesis was explored that a state of hyperglycemia evoked by strong emotion existed at the time of the test. Certainly the circumstances under which the urine specimens were being secured were conducive to apprehension and emotional excitement. Blood specimens for assay were accordingly drawn as soon as the urine was found positive—never more than 15 to 30 minutes after voiding. The blood was collected on the medical examining line, just ahead of the room where inoculations were being given. *No unusually high blood sugar levels were discovered under these emotion-provoking circumstances.* With 10 specimens the sugar content was between 73 and 100 mg. per 100 ml.; in the remaining 4 it fell between 120 and 148 mg. per 100 ml. This absence of striking rise in blood sugar in humans

the readings in any subject rose above 180 mg. per 100 ml., which is the critical value accepted commonly as top normal³ for subjects not on carbohydrate-poor intakes. Exton-Rose tests on a series of non-dextro-uric controls yielded essentially identical results. These results with the dextrose tolerance tests are identical with those described earlier in this report, and suggests that a state of temporarily impaired glucose metabolism existed in many of the men at the time of their arrival.

Interviews brought out that the lives of most of the men had been out of balance in the final few days of civilian life. With regularity one learned of hasty meals, farewell parties, gross lack of sleep, alcoholic drinks, family strains, long trips by train or bus, and anticipatory anxiety and

TABLE 4.—RELATIONSHIPS BETWEEN DIAGNOSIS AND CONCENTRATION OF SUGAR IN INITIAL URINE SPECIMENS

	No. specimens	0.5% or less	0.6% to 1%	1.1% to 2%	Above 2%
Transitory	225	113 (50%)	70 (31%)	36 (16%)	6 (3%)
Low renal threshold	19	7 (36%)	6 (32%)	6 (32%)	0
Renal glycosuria	7	2 (29%)	3 (43%)	1 (14%)	1 (14%)
Diabetes mellitus	21	3 (14%)	5 (24%)	4 (19%)	9 (43%)
Undiagnosed	52	21 (40%)	15 (29%)	13 (25%)	3 (6%)
Totals	324	146 (45%)	99 (31%)	60 (18%)	19 (6%)

undergoing emotional reactions is in line with the experience of other workers who have studied this problem.⁶

Subsequent tests showed that only 1 of these 14 subjects had a renal threshold below 160 mg. per 100 ml. blood level. This individual "spilled over" at 133 mg. per 100 ml., yet when his urine was positive on the day of arrival his blood level had been only 75 mg. per 100 ml.

Exton-Rose dextrose tolerance studies were performed with all but 1 man on the morning following the urine analysis. In 5 the venous blood sugar level rose to 200 mg. per 100 ml. or higher in either the first or the second $\frac{1}{2}$ hour after ingestion of the dextrose. The tolerance tests were then repeated after feeding the subjects liberal amounts of carbohydrate for several days. On repetition not one of

excitement. Yet further query brought out that all these emotion stimulating situations held to as great a degree for trainees with sugar-free urines as for those who were voiding sugar.

Concentration of Sugar in the Urine. The possible relationships between the amount of sugar in the initial urine and the diagnoses ultimately arrived at were evaluated (Table 4). Such wide scatter of the individual readings was found that knowledge of the quantity of sugar in any single specimen of urine would appear of little service as a diagnostic aid. One notes, however, that about half of the transitory cases had sugar concentrations of 0.5% or less, which is the "trace" zone of readings, whereas only a minority of the low renal threshold, renal glycosuria and diabetes mellitus patients exhibited

such weak concentrations. Conversely, of the diabetes patients nearly half had more than 2% sugar in their initial specimens, as opposed to a much lower incidence for the other groups.

Summary. 1. A survey was made of the nature and incidence of melituria as encountered in 77,293 consecutive healthy males at the time of their arrival at a Maritime Service Training Station. Of these, 225 (0.29%) were found to have transitory benign melituria; 19 (0.02%), low renal threshold; 7 (0.01%), persistent renal glycosuria; 52 (0.07%) were undiagnosed; and 21 (0.03%) had diabetes mellitus which had escaped detection in the enrolling offices. No instances of congenital non-dextrosic melituria were recognized.

2. The transitory form of melituria tended to become more frequent with advance in age.

3. The mechanism which provoked the transitory melituria in some subjects on their 1st day of Maritime Service training were far from clear, but emotional influences, fatigue, inflexibility of metabolic adjustments, and poor "carbohydrate saturation" must have played rôles of inconstant and differing importance. The blood sugar was not elevated in many of the subjects at the hour when their urines were positive for sugar.

4. The concentration of the sugar in the urine proved of little aid as a guide to diagnosis.

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THE REACTION OF THE VESSELS OF THE MESENTERY AND INTESTINE TO ANGIOTONIN AND RENIN*†

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It is well known that one of the factors responsible for hypertension is increase in peripheral resistance to blood flow. Recently we have shown¹ that in hypertension of renal origin, the arteries of the rabbit's ear constrict and remain persistently constricted, indicating an increase in peripheral resistance in the somatic area. A similar constriction occurs in acute hypertension produced by injections of angiotonin and renin.² The present experiments were performed to find out by direct observation whether or not such constriction occurs in the splanchnic area following injections of these substances, and if so, whether it is of the same degree as that which has previously been shown to occur in the somatic area.

Methods. Four rabbits were used, and in each a portion of the intestine, usually jejunum or ileum, and its attached mesentery was exteriorized in a transparent intestinal mesenteric chamber,¹⁰ using sterile technique. During insertion of the chambers the rabbits were anesthetized with pentobarbital (30 mg./kg. of body weight) injected subcutaneously. The chambers were protected by special shields when not being studied, and remained permanently attached to the rabbit. Intravenous injections of angiotonin and of renin were made 1 to 14 days after insertion of the chambers, and the effects upon the vessels of the mesentery and intestine studied by direct microscopic observation. The arteries and veins were studied at magnifications of from 10 to 30 diameters. The capillaries were studied

at magnifications of 200 and 400 diameters, and only those in the mesentery were observed. Ocular micrometric measurements of the vessels were made during control periods of 15 to 30 minutes before injection and the fields were photographed. Following the control observations, angiotonin and renin were injected and the same vessels were again measured and photographed. All injections were made into lateral ear veins.

The arteries of the mesentery studied had control diameters of from 0.12 to 0.42 mm., and those of the intestine 0.09 mm. The veins of the mesentery had control diameters of from 0.3 to 0.9 mm. Those of the intestine were not studied.

The renin used was hog renin and was prepared by the method of Helmer and Page.³ The angiotonin was prepared by the method of Page and Helmer.⁴

Observations. CONTROL. With the exception of rhythmic and spontaneous constriction of the precapillary sphincters and metarterioles, such as described by Chambers and Zweifach,⁴ no appreciable changes were observed in the diameters of any of the vessels.

EXPERIMENTAL. 1. *Reaction of the Vessels to Angiotonin.* Fifteen injections of angiotonin were made in amounts varying from 0.2 to 0.5 cc., and the effects upon the arteries, veins and capillaries studied.

Arteries: Fourteen arteries of the mesentery and 2 arteries of the intestine were observed. The arteries all responded similarly. Between 1 and 2 minutes following

* This work was aided by a grant made to the department of anatomy at the University of Pennsylvania by Eli Lilly & Company.

† Brief reference to some of the results given in this paper was made in a report presented before the Physiological Society of Philadelphia, session of May 19, 1942 (AM. J. MED. SCI., 203, 914, 1942), and in an abstract prepared for the American Association of Anatomists (Anat. Rec., 85, 330, 1943).

the injections, they constricted 25 to 50 % of their control diameters, and returned to their control diameters within 3 to 10 minutes; usually within 3 to 6 minutes after the smaller injections and 5 to 10 after the larger ones.

Veins: The degree of constriction in the veins was not as great as in the arteries. It varied from 10 to 30 % of the control diameters. The time of onset of such constrictions was usually between 1 and 4 minutes after the injections. Relaxation did not occur until some minutes later, but the exact time of return of the veins to their normal calibers was not determined.

Typical constriction of the mesenteric arteries and veins caused by injection of angiotonin is shown in Plate I.

Capillaries: The capillaries did not respond visibly. They did not constrict, their lumens remaining open at all times. No appreciable changes were seen in rate of flow through the capillary bed following injections of 0.2 and 0.3 cc. of angiotonin. The injection of 0.5 cc. may have produced a slight reduction in rate of flow, but in any case the blood flow was not interrupted at any time.

2. Reaction of the Vessels to Renin. Thirteen injections of renin were made in amounts varying from 0.1 to 0.4 cc., and the effects upon the arteries, veins and capillaries studied.

Arteries: Thirty-two arteries of the mesentery and 3 arteries of the intestine were observed. The arteries all responded similarly; they constricted 40 to 70 % of their control diameters after 1 to 2 minutes, and returned to their control diameters after 15 minutes. Thus the constrictions caused by injections of renin lasted in some cases longer than those caused by angiotonin.

In 3 experiments the vessels of the mesentery and intestine were watched with a binocular dissecting microscope at a magnification low enough to permit seeing all of them at once. Following injections of 0.2 cc. of renin all of the arteries

were seen to constrict at about the same time.

Veins: The veins also constricted following injections of renin. The degree of constriction varied from 12 to 40 % of the control diameters.

Capillaries: The capillaries did not respond visibly. No appreciable changes in rate of blood flow through the capillary bed were observed following injections of 0.1 to 0.2 cc. of renin. Whether or not some reduction in rate of flow followed injection of larger amounts we are not certain. It was possible to determine by direct observation, however, that the blood flow was not interrupted at any time.

3. Effect of Angiotonin and Renin Upon the Motility of the Intestine. Injections of both angiotonin and of renin were followed by vigorous peristalsis of the intestine. Such peristalsis usually began during the 2nd minute after the injection and lasted for about 2 to 3 minutes. This peristalsis caused swaying movements of the mesentery, and in consequence some change in arrangement and position of the vessels in the chambers (see Plate I).

In some instances peristalsis was observed during the control periods, and in these cases it was seen that although the vessels of the mesentery and intestine were moved about by it, they did not constrict. Consequently the constriction of the arteries and veins observed in the present experiments was evidently due to the effect of the angiotonin and renin upon the vessels themselves, and not to the movements of the intestine and mesentery.

Discussion. It is obvious from the data given above that the arteries and veins of the mesentery and the arteries of the intestine of unanesthetized rabbits constrict in response to angiotonin and renin when these substances are injected intravenously. The degree and duration of arterial constriction following the injections of angiotonin and renin were approximately the same as in transparent moat chambers in rabbits' ears² following injections of the same amounts of these

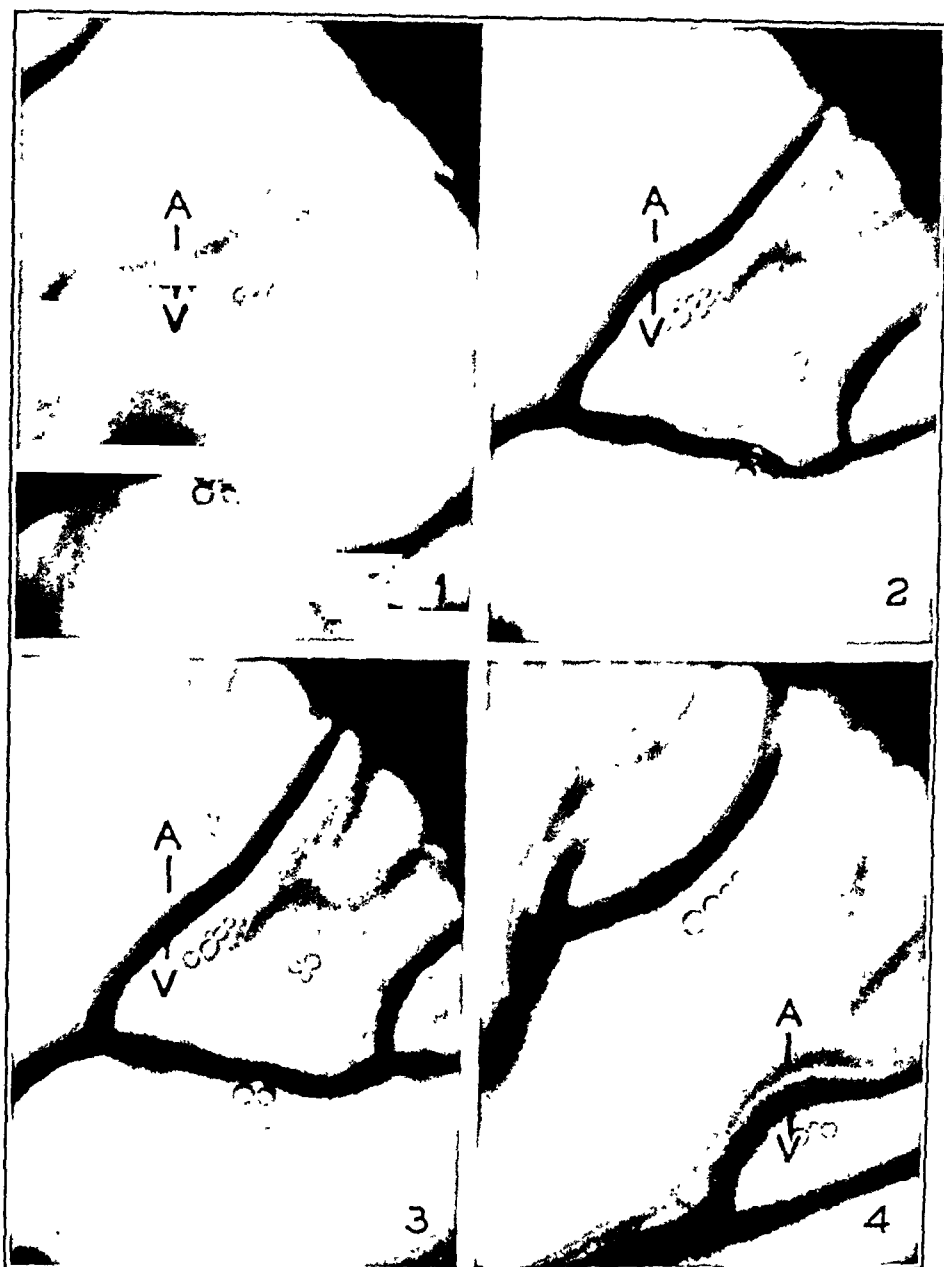


PLATE I.—Photographs of arteries and veins of the mesentery of an unanesthetized rabbit, showing the appearance of the vessels in an intestinal mesenteric chamber before and after intravenous injection of 0.5 cc. of angiotonin. The same artery is indicated by "A" and the same vein by "V" in all of the photographs. The change in positions of the vessels was caused by movements of the mesentery due to the violent peristalsis of the intestine which followed the injection. ($\times 5.0$) 1, Before injection. 2, The same vessels 1½ minutes after the injection. The arteries and veins are constricted. 3, The same vessels 2½ minutes after the injection. This was the time of maximal arterial constriction. 4, The same vessels 12 minutes after the injection. The arteries and veins have relaxed to approximately their control diameters.

substances. That the blood flow remained approximately normal in the mesenteric and intestinal vessels in the present experiments following injections of 0.1 to 0.2 cc. of renin and of 0.2 to 0.3 cc. of angiotonin was shown by direct microscopic observations of the flow through the capillary bed, and by absence of blanching of the mesentery and intestine.

The above observations are in accord with the suggestion made by Landis,⁶ based upon studies by Landis, Montgomery and Sparkman,⁷ that "in contrast to other pressor substances the active material in kidney extracts increases the tone of all the arterioles throughout the body more or less equally. Since in each vascular bed peripheral resistance and arterial pressure rise proportionally, blood flow remains approximately normal."

Constriction of the veins in the present experiments was anticipated because it had been observed in the rabbit's ear following injections of renin and angiotonin,² and because veins of the size studied contain appreciable amounts of muscle. Furthermore, it had been shown that angiotonin and renin cause constriction of arterioles lacking a nerve supply, and hence presumably angiotonin has a direct action upon the vascular muscle. It is of interest in this connection that Wilkins and Duncan⁹ observed a rise in venous pressure in normal persons in which hypertension had been induced by injection of angiotonin.

The present studies demonstrate constriction of arteries in the living animal following injection of renin and angiotonin. Previous studies by Bingle and Strauss³ show that the isolated beef artery

likewise constricts upon addition of active kidney extracts.

It has been reported previously that the capillaries in transparent chambers in rabbits' ears do not constrict following injections of angiotonin and renin,² and the present studies show that in the mesentery they likewise fail to constrict.

Since the arteries of the mesentery and intestine constrict following injections of angiotonin and renin, it seems not unlikely that they constrict also during chronic hypertension of renal origin. As stated previously, during the development of hypertension of renal origin the arteries in the rabbit's ear constrict, and remain persistently constricted during the period of hypertension, thus increasing the resistance to blood flow. The present work suggests that during such hypertension, arterial constriction in the splanchnic area may also play a part in increasing the resistance to blood flow.

Summary. 1. The present experiments demonstrate by direct microscopic observation that both angiotonin and renin, when injected intravenously, cause constriction of the arteries of the mesentery and intestine of unanesthetized rabbits. The veins of the mesentery also constricted. The veins of the intestine were not studied. Neither substance caused constriction of the capillaries, nor any appreciable change in rate of blood flow, as shown by direct observation of the capillary bed; nor did either substance produce blanching of the mesentery and intestine.

2. Both angiotonin and renin, injected intravenously, caused vigorous peristalsis of the intestine.

We are indebted to Mr. B. B. Varian for the photographs.

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SULFAPYRAZINE—ITS USE IN THE PROPHYLAXIS OF RESPIRATORY DISEASE

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CONDITIONS peculiar to military operations frequently have operated to produce excessively high rates of respiratory disease in general and of streptococcal disease in particular. In many instances the loss of man-days caused by this type of disease had become such a serious barrier to the effective prosecution of the war effort that it became expedient to attempt to devise procedures that might effectively lower these disease rates. For this reason, and based on assumptions implicit in the literature,^{3,4,5} the prophylactic use of small daily dosages of sulfonamides for the control of respiratory diseases has been tried and has met with considerable success in the desired temporary goal.

Of the sulfonamides that have been chosen for this purpose, sulfadiazine has been the drug of almost universal choice. Numerous experiences have attested to the fact that its use has been attended not only with success in lowering the respiratory disease rate but also with a surprisingly low incidence of serious toxic reactions.^{3,4,5} Unfortunately, however, during the winter of 1944-1945, strains of Group A, Type 17 streptococcus highly resistant to sulfadiazine were recognized with increasing frequency and were responsible for relatively alarming outbreaks of streptococcal diseases. As might be expected, the use of sulfadiazine for either prophylactic or therapeutic purposes proved ineffective in controlling diseases due to this group of organisms.

The mechanism of acquired resistance by an organism to a chemotherapeutic agent is as yet unclarified. However, contrary to previous ideas gained largely

through a study of "sulfonamide fastness" induced *in vitro*, preliminary observations⁴ have indicated that organisms naturally resistant to 1 type of sulfonamide may retain their sensitivity to other sulfonamide drugs. If these observations prove to be true, it might be possible effectively to employ 1 sulfonamide for prophylaxis against organisms resistant to the prophylactic activity of another sulfonamide drug. Or it appears conceivable that, if prophylaxis with one type of sulfonamide is accompanied by hospital admissions due to disease caused by organisms resistant to this particular drug, another type of sulfonamide might be used for the treatment of such patients.

Moreover, whereas sulfadiazine prophylaxis has been repeatedly demonstrated to be both effective (in the absence of sulfadiazine-resistant strains) and relatively non-toxic, it is possible that other sulfonamide drugs might be used with even greater effectiveness and with less dangerous side reactions. Accordingly, it appeared important to evaluate the results obtained with the use of other sulfonamides for the chemoprophylaxis of respiratory infections.

Preliminary clinical studies have indicated that sulfapyrazine^{4,6,7} was effective in the treatment of pneumococcal disease and that its use was attended by a very low rate of toxic reactions. Whereas a relatively small number of patients had been treated successfully with therapeutic dosages over short periods of time, to our knowledge this drug has never been used for chemoprophylaxis and no data were available concerning its effects on man

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when given in daily small dosages over an extended period of time. It therefore appeared desirable to acquire data concerning sulfapyrazine prophylaxis. Accordingly, the effectiveness of sulfapyrazine for the chemoprophylaxis of respiratory disease was investigated.

Methods. This study was performed at an Army Air Base during the winter months of 1944-45. At this Field, large groups of soldiers received a 26 week course of instruction and it was therefore possible to study respiratory disease rates in a comparatively stable population over a significantly long period of time. Moreover, due to its location and climatic conditions, this Field had experienced, in the past, relatively high respiratory disease rates and it was also known, from previous studies, that a Group A, Type 17 hemolytic streptococcus had long been endemic.

From the population of this Field, 4 separate squadrons, each containing approximately 1000 men, were chosen to receive prophylaxis. Two of these squadrons, Squadrons H and L were located in one area of the Field. The other 2, Squadrons N and O were both located in a widely separated region of the same Field. All 4 squadrons were quartered in similar cantonment type wooden barracks. The personnel of Squadrons H and L used the same mess, classrooms, theater, etc., so that a necessary amount of intermingling occurred. The same was true for the personnel of Squadrons N and O. However, the administrative routine of the Field was such that no intermingling occurred between the personnel of Squadrons H and L, on the one hand, and the personnel of Squadrons N and O, on the other hand. For the purposes of this investigation, only student personnel of these 4 squadrons were studied as the general environmental and living conditions of the attached permanent party personnel were not comparable.

Since this study was designed to compare the relative effectiveness of sulfapyrazine with sulfadiazine and since, for the reasons

enumerated above, Squadron L was regarded as comparable to Squadron H and Squadron N comparable to Squadron O, Squadrons L and O were chosen to receive prophylaxis with sulfapyrazine whereas Squadrons H and N received sulfadiazine. It was believed that it would thus be most feasible to compare the relative activities of the 2 different drugs.

The respiratory disease rates were determined for all 4 squadrons during a 4 week control period. At the end of that time, Squadron H was given sulfadiazine and Squadron L sulfapyrazine prophylaxis for a 30 day period. Ten days later, Squadron N was started on sulfadiazine and Squadron O on sulfapyrazine prophylaxis for the following 30 days. The personnel of all 4 squadrons each received 1 gm. per day of the appropriate drug.

The administration of the drug was accomplished with the excellent coöperation of the Squadron Commanders, who made it a part of the Barracks Chiefs' duties to administer the daily dosage and to certify that it was actually swallowed while in their presence. A negligible number of men was excused from taking the drugs due to a previous history of sulfonamide sensitivity. Numerous unannounced spot checks were made using the simple so-called "newspaper test"² which was capable of detecting even very minute quantities of sulfonamides in the urine. Blood specimens drawn at intervals from representative samples of the squadrons showed traces only of sulfapyrazine, but since our only object was to determine whether or not the drug was being taken, we were satisfied with the results of the urine test. On only 1 or 2 occasions as indicated by this test was anyone found who had not been taking the drug.

A number of pertinent facts were recorded and analyzed: 1. Complete records were kept of all hospital admissions for acute respiratory disease.*

2. In order to ascertain the influence of prophylaxis on streptococcal disease in particular, throat cultures were routinely taken on all hospital admissions within a maximum

* In this study acute respiratory disease was used to include both upper and lower respiratory infections, including nasopharyngitis (both bacterial and non-bacterial), laryngitis, tonsillitis, scarlet fever, bronchitis, pneumonia (bacterial and non-bacterial) influenza and other virus infections of the respiratory tract. It did not include any chronic infections such as chronic bronchitis, tuberculosis, chronic sinusitis, etc. In addition re-admission for complications of acute respiratory disease such as mastoiditis, otitis media, etc., were not considered as separate admissions.

of 15 hours after their entrance to the hospital wards and prior to any treatment.

3. Hospital admission rates reflected the trends of relatively severe and disabling respiratory disease. On the other hand, numerous personnel suffered from mild infections not considered severe enough for their hospitalization. It was felt accordingly that a somewhat more revealing study of the trend of respiratory disease rates could be accomplished by recording all dispensary

were taken throughout the control period and for the duration of the prophylaxis in the squadrons included in this study.

5. All personnel receiving prophylaxis was carefully watched for any evidences of drug reactions. All men had been instructed to report immediately to the dispensaries at the first signs of any of the symptoms known to occur with the use of sulfonamides. Those individuals who developed any possible symptoms of reaction were removed tem-

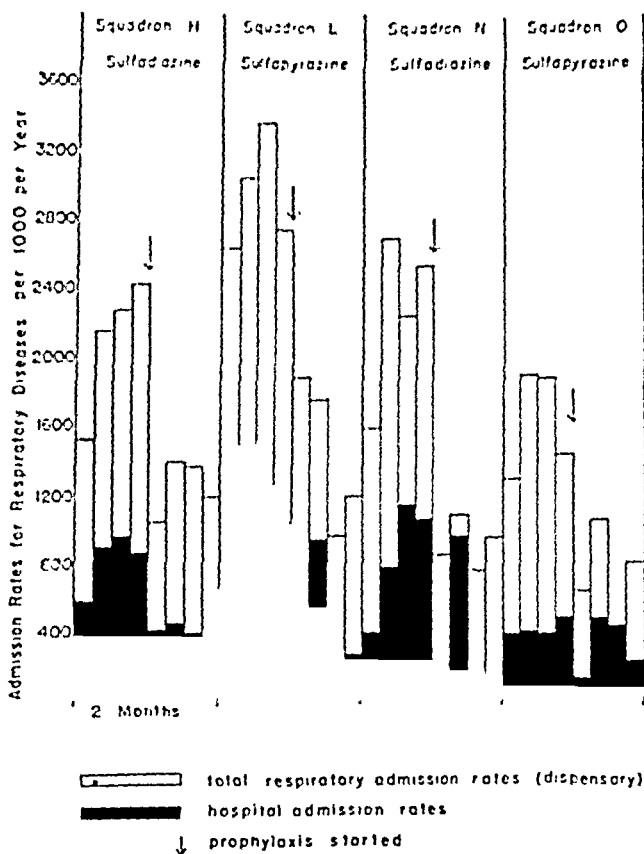


FIG. 1.—Effects of sulfapyrazine and sulfadiazine prophylaxis on respiratory disease rates by weeks.

admissions seeking aid for respiratory disease. Accordingly a tabulation was kept on all dispensary admission rates for respiratory disease. In this study, therefore, the dispensary admissions were used as an index of total respiratory diseases.

4. In order to determine the effect of prophylaxis on carrier rates among the healthy personnel, weekly throat cultures

porarily, at least, from further prophylaxis and were referred to the dermatologist for appropriate study. The latter recorded the type of reaction, its severity and duration and determined the ability of the patient to react to further small doses of the same or different sulfonamide.*

* We are indebted to Major Ben A. Newman, M.C., for his invaluable aid in these particular studies.

Results. A. Effect of Respiratory Disease Rates. The effects of the prophylaxis on respiratory diseases were evidenced by a comparison of the admission rates for both hospital and dispensaries during the control period preceding prophylaxis and for the 30 day period of prophylaxis. These figures revealed that the institution of both sulfapyrazine and sulfadiazine resulted in a marked and abrupt decline in the rates for all acute respiratory diseases. A graphic comparison of these rates is given in Figures 1 and 2.

sion rates were comparatively low both before and during prophylaxis and the differences resulting from prophylaxis did not appear significant.

However, as noted above, hospital admissions reflected only moderately severe and severe cases. A somewhat more accurate picture of the effects of prophylaxis was obtained through comparison of the dispensary admission rates for respiratory diseases inasmuch as these latter figures more closely revealed the total number of respiratory infections. These

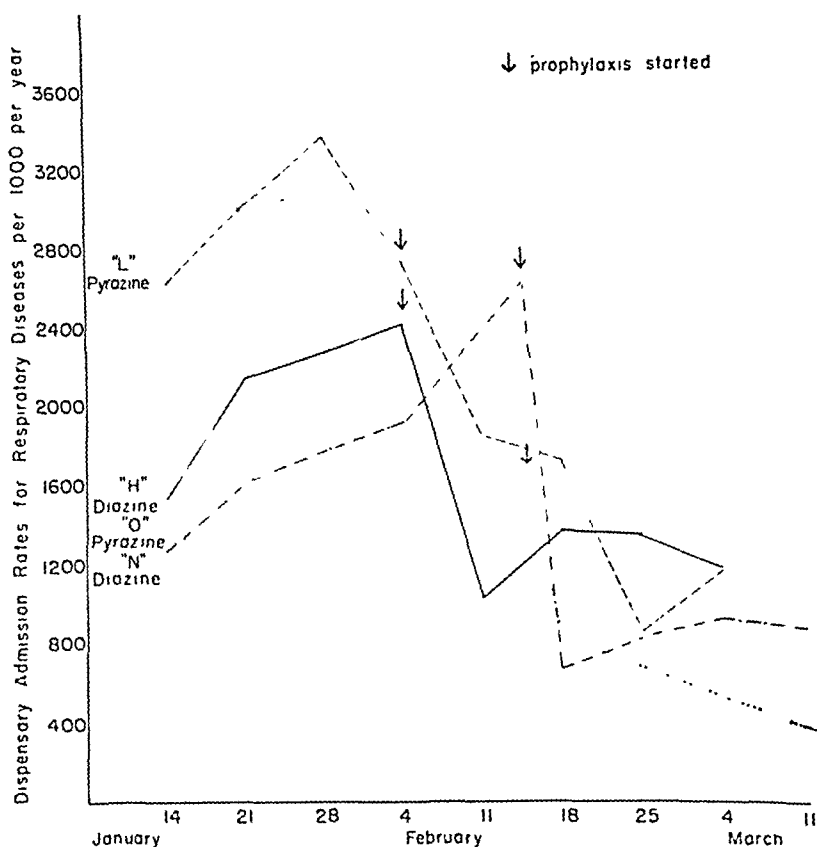


FIG. 2.—Effects of prophylaxis on respiratory disease rates in four squadrons.

Figure 1 demonstrates a marked and uniform drop in hospital admission rates in Squadron H during the period of sulfadiazine prophylaxis. In Squadron L (sulfapyrazine) and Squadron N (sulfadiazine) the decreases in hospital admission rates were also marked although, in each instance, during the 2nd week of prophylaxis, there was a temporary rise in these rates. In Squadron O, which received sulfapyrazine, the hospital admis-

figures for dispensary rates are also given in Figures 1 and 2 and demonstrate that all 4 squadrons witnessed a marked decline in respiratory disease coincident with the inception of both sulfapyrazine and sulfadiazine prophylaxis.

Numerous facts tend to substantiate the conclusion that the decline in respiratory disease coincident with prophylaxis could be attributed solely to the effects of the latter and not to the spontaneous disap-

pearance of an epidemic. In the first place, studies from previous years had established the fact that the epidemic period of respiratory disease at the Field did not end spontaneously until May, whereas the decline witnessed during this particular investigation occurred in a season (February and March) during which the rates could be predicted to increase or remain at their already high levels. In the second place, prophylaxis was instituted in 2 of the squadrons 10 days after its inception in the other 2. Figure 2 demonstrates that the rates in the 2 squadrons not yet receiving prophylaxis continued to remain at high levels during this interval, whereas the rates for the other 2 squadrons had already declined. However, immediately after the institution of prophylaxis in the last 2 squadrons, the respiratory disease rates for these groups experienced the same sharp decrease. Again, while not conclusive, these facts bring additional weight to the argument that the declines in respiratory diseases were not spontaneous.

the 2 squadrons receiving sulfapyrazine prophylaxis are compared with the rates, first for 4 other squadrons and, secondly, for the entire remainder of the post.

It was not to be expected that these latter rates would be strictly comparable to those of the squadrons receiving prophylaxis. Due to the plan for operation of the Field, no squadrons other than the ones chosen to receive prophylaxis could be selected to form a comparable epidemiologic unit.* Due to different duties, different housing conditions, different durations of stay on the Field, etc., conditions in the 4 untreated squadrons were such as to result in respiratory disease rates somewhat lower than those found in the squadrons receiving prophylaxis. Furthermore, the total of those units comprising the entire remainder of the post consisted largely of well-seasoned personnel with duties and housing conditions entirely different from those of the 4 experimental squadrons.

It is recognized that epidemics originate and are initially confined to a single bar-

TABLE 1.—COMPARISON OF SULFADIAZINE AND SULFAPYRAZINE PROPHYLAXIS

Respiratory disease rates*	Squadron* receiving sulfadiazine		Squadrons receiving sulfapyrazine	
	4 wks. preceding prophylaxis	4 wks. during prophylaxis	4 wks. preceding prophylaxis	4 wks. during prophylaxis
Dispensary admissions	2139	1093	2176	1048
Hospital admissions	807	364	655	391
Average population	2030	2070	1840	1860
Untreated Controls				
	4 Squadrons†		Remainder of Post†	
Hospital admissions	459		282	312
Average population	4360		Restricted	Restricted

* Rates expressed in terms of admission* per 1000 per year for the 4 week period under consideration.

† Figures not available for dispensary admissions.

A third point of evidence consisted in a comparison between the admission rates for respiratory diseases in the experimental squadrons and in other squadrons not chosen to receive the benefit of prophylaxis. Those figures are found in Table 1 wherein the hospital admission rates for respiratory disease in the 2 squadrons receiving sulfadiazine prophylaxis and in

rack and then spread through an entire squadron. Due to the quantitative differences in exposures of members of other squadrons from those of the affected squadron, rates for respiratory diseases might be expected to vary. These quantitative differences were such as to maintain respiratory disease rates among the 4 untreated squadrons at somewhat lower,

* The ideal method to establish control units for those receiving prophylaxis would have been to divide each squadron into 2 halves and to have reserved one of these halves for prophylaxis while the other half received none. Unfortunately, inter-barracks shifts of personnel within the same squadron were so necessary that, according to the Squadron Commanders, such controls could not be maintained.

and for the entire remainder of the post, at markedly lower figures than those found in the squadrons chosen to receive prophylaxis. Nevertheless, a necessary amount of intermingling occurred between personnel of the squadrons receiving prophylaxis and the personnel of the remainder of the post. Accordingly, any natural trends affecting the respiratory disease rates in 1 group of squadrons would be expected to be reflected sooner or later in the other groups provided the comparison was made over a significantly long period. The figures in Table 1 indicate, however, that whereas a markedly significant decrease in respiratory disease rates occurred in the experimental squadrons during the period of prophylaxis, rates for the remainder of the Field were such as to warrant the conclusion that the marked decreases in respiratory disease rates observed in Squadrons H, L, N and O were not due to any spontaneous, cyclic variations.

strated that not only streptococcal diseases but also that other respiratory diseases, even those thought to be caused by agents not susceptible to sulfonamides, were decreased as a result of prophylaxis. The explanation for this fact is not clear. However, in this particular study, the same findings were observed. The use of sulfonamides was expected to produce its greatest effect on those diseases caused by organisms sensitive to these drugs. Such diseases include all those caused by the beta-hemolytic streptococcus and the gonococcus.* As a result of the routine throat culture studies on all hospital admissions, it was possible to differentiate the respiratory diseases of non-streptococcal origin from those caused by the streptococcus.

In Table 2 a comparison is made of the effects of both sulfapyrazine and sulfadiazine prophylaxis on the admissions due to organisms definitely susceptible to sul-

TABLE 2.—COMPARISON OF SULFAPYRAZINE AND SULFADIAZINE PROPHYLAXIS ON DISEASES CAUSED BY STREPTOCOCCI AND GONOCOCCI AND DISEASES NOT REGARDED SUSCEPTIBLE TO SULFONAMIDE THERAPY

Types of disease	Sulfadiazine		Sulfapyrazine	
	4 wks. preceding prophylaxis*	4 wks. during prophylaxis*	4 wks. preceding prophylaxis*	4 wks. during prophylaxis*
Total cases caused by streptococcus and gonococcus	29	10	34	11
Strep. pharyngitis	13	5	10	5
Scarlet fever	11	2	16	2
Gonorrhea	5	3	8	4
Non-streptococcal respiratory disease	102	51	71	49
<i>Untreated Control—Remainder of Post</i>				
		4 wks. preceding prophylaxis*	4 wks. during prophylaxis*	
Scarlet fever		39	80	
Gonorrhea		57	59	

* Figures represent actual number of cases.

Accordingly, by virtue of the various arguments advanced above, it is believed that the weight of the evidence leads to the conclusion that the decline in respiratory disease rates in the squadrons receiving prophylaxis could be attributed quantitatively to the use of chemoprophylaxis.

In previous experiences with sulfonamide prophylaxis^{3,4,5} it has been demon-

strated that not only streptococcal diseases but also that other respiratory diseases, even those thought to be caused by agents not susceptible to sulfonamides, were decreased as a result of prophylaxis. The explanation for this fact is not clear. However, in this particular study, the same findings were observed. The use of sulfonamides was expected to produce its greatest effect on those diseases caused by organisms sensitive to these drugs. Such diseases include all those caused by the beta-hemolytic streptococcus and the gonococcus.* As a result of the routine throat culture studies on all hospital admissions, it was possible to differentiate the respiratory diseases of non-streptococcal origin from those caused by the streptococcus.

* No cases of meningitis were observed during the entire period of study. Sulfonamide prophylaxis in the dosages used here, did not appear effective against pneumococcal pneumonia.

than those enumerated above also was decreased coincident with the use of both types of prophylaxis. Table 2 also demonstrates that the incidence of streptococcal disease and of gonorrhea underwent no spontaneous decline in the remainder of the Field.

in diseases caused by the streptococcus and gonococcus ($t = 2.71$). On the other hand, there was no significant difference between sulfapyrazine in Squadron L and sulfadiazine in regard to reduction of hospital admission rates for all acute respiratory diseases ($t = 1.05$).*

TABLE 3.—TABLE OF DIFFERENCES FOR 4 WEEKS PRIOR TO AND 4 WEEKS DURING PROPHYLAXIS

	2 diazine squadrons	Squadron O pyrazine	Squadron L pyrazine	4 untreated control squadrons
Total hospital respiratory disease admissions	68	9	32	14
Total hospital streptococcal respiratory disease admissions	17	2	17	7
Total gonorrhea cases	2	1	3	•
Total respiratory disease dispensary admissions	160	69	89	•
Average population	2050	1018	833	4253

* Figures not available.

A statistical analysis of the various results enumerated above was kindly performed by Lt. Arthur Kemp, A.C., of the Department of Statistics, AAF School of Aviation Medicine. Table 3 gives the differences between 4 weeks prior to and 4 weeks during prophylaxis for total (dispensary) respiratory diseases, hospital admissions for respiratory diseases and hospital admissions for streptococcal disease plus gonorrhea. It was found that both squadrons receiving sulfadiazine prophylaxis were comparable and could be used as a single unit for statistical comparison. However, for reasons enumerated above, the 2 squadrons which received sulfapyrazine prophylaxis were not found to be comparable. Accordingly, it became necessary to compare each of the squadrons receiving sulfapyrazine with the other groups.

The method used consisted of an analysis of the significance of the differences between the proportions of admissions of the several kinds to their respective populations.

These figures reveal that when the rates for Squadron L (sulfapyrazine) were compared with those for both squadrons receiving sulfadiazine, sulfapyrazine was significantly more effective than sulfadiazine in producing a decrease in dispensary admission rates ($t = 2.82$) and a decrease

However, a comparison of the rates for the other squadron receiving sulfapyrazine (Squadron O) with those for the 2 squadrons receiving sulfadiazine shows no significant differences between sulfapyrazine and sulfadiazine in regard to reduction of dispensary admission rates for respiratory disease ($t = 1.20$) and for diseases caused by the streptococcus and gonococcus ($t = 1.9$). On the other hand, as expected, sulfadiazine was significantly more effective than sulfapyrazine in Squadron O in regard to reduction of hospital admission rates for acute respiratory diseases ($t = 4.18$).

These analyses give somewhat conflicting results between the 2 squadrons receiving sulfapyrazine and those partaking of sulfadiazine prophylaxis. It therefore must be concluded that the results of this study failed to produce evidence of the relative superiority of either sulfadiazine or sulfapyrazine for the prophylaxis of respiratory diseases. It is obvious that future studies involving much larger groups are required for the solution of this problem.

Those cases of streptococcal disease which were admitted to the hospital from the squadrons receiving both sulfapyrazine and sulfadiazine prophylaxis proved principally to be due to a Group A, Type 17 streptococcus. Sensitivity stud-

* t = the critical ratio of the difference between 2 proportions divided by the standard error of the difference. In order to be significant at the 1% level, *i. e.*, in order to have less than 1 chance in 100 that a difference as large as that observed is due to chance, the value of t must exceed 2.58.

ies were performed on these organisms with somewhat conflicting results inasmuch as a number of them proved to be sensitive to both sulfonamides whereas others were relatively resistant. No detailed analysis is appended but sulfapyrazine and sulfadiazine appeared to be about equally ineffective in controlling disease caused by this particular organism.

An analysis of the carrier rates is beyond the scope of this paper because of the presumed relationship between carrier rates and resistance to the drug. For this reason it will be sufficient to mention only that the carrier rates in the squadrons receiving prophylaxis did not recede sharply following its introduction.

pyrazine reactions belonged to this category and presented manifestations lasting from 3 to 11 days. In this group there was 1 urticarial reaction, 1 morbilliform eruption and 1 case of erythematous patches in areas of lichenification which had resulted from a former drug reaction. All 3 of these individuals had taken sulfadiazine at some previous period and 2 of these had experienced reactions to this drug at an earlier date. The patient with urticaria, however, failed to recall a previous sulfonamide reaction.

Of the 7 men reacting to sulfadiazine, 4 showed cutaneous manifestations, 1 had nausea and diarrhea, 1 presented a transient neutropenia accompanied by

TABLE 4.—TOXIC REACTIONS TO SULFONAMIDE PROPHYLAXIS

Drug used	Type of reaction	Appearance in days after start of prophylaxis	Duration of symptoms	Previous reactions to sulfonamides	Remarks
Sulfapyrazine	Cutaneous—urticarial	1	3	None	Previous urticaria to A.P.C. capsules
Sulfapyrazine	Cutaneous—morbilliform	4	10	Yes	Fever and chills with diazine prophylaxis 1 year previous
Sulfapyrazine	Cutaneous—fixed drug reaction	7	5	Yes	Similar previous reaction to diazine 1 year previous
Sulfadiazine	Cutaneous—fixed drug reaction	1	7	None	Readministration of 1 gm. diazine reproduced reaction; 1 month later tolerated 7 daily gm. doses of sulfapyrazine
Sulfadiazine	Cutaneous—erythema multiforme	16	5	None	Readministration of 1 gm. daily of pyrazine for 3 days reproduced rash
Sulfadiazine	Cutaneous—scarlatiniform	1	13	None	
Sulfadiazine	Cutaneous—urticarial	8	6	None	Readministered pyrazine 1 gm. daily for 6 days with no reaction
Sulfadiazine	Conjunctivitis	1	5	Yes	Previous similar reaction to diazine 1 year ago
Sulfadiazine	Granulopenia, headaches	5	10	None	Recovered spontaneously; not very ill
Sulfadiazine	Nausea, cramps, diarrhea	7	4	None	Readministration of diazine produced same reaction after 6 days; pyrazine 1 gm. per day for 7 days was tolerated without reaction

B. Toxic Manifestations. Approximately 2000 men received 1 gm. daily doses of either sulfapyrazine or sulfadiazine for a total duration of 30 days. The total number of reactions observed in both groups and the types of reactions are given in Table 4. It is apparent that there was a total of 10 reactions, 7 in the sulfadiazine and 3 in the sulfapyrazine treated group. In accord with other experiences with sulfonamide prophylaxis, serious reactions were conspicuous by their absence.

The majority of reactions (Table 4) were cutaneous in type. All of the sulfa-

headache and 1 displayed a conjunctivitis. The case of neutropenia improved spontaneously, the neutrophils increasing from 15 to 60% in 7 days. The individual developing conjunctivitis a few hours after his initial dose of sulfadiazine reported a similar episode following the previous use of sulfadiazine.

The time for the development of these reactions varied considerably. The case of conjunctivitis mentioned above occurred a few hours after the initial dose and the longest interval between start of

prophylaxis and appearance of the reaction was 16 days.

Three of the individuals reacting to sulfadiazine were given trial doses of sulfapyrazine after the initial symptoms had disappeared. One case, involving a scarlatiniform eruption, had a similar reaction after 3 gm. daily of sulfapyrazine. The other cases (urticaria and nausea) were able to take 6 and 7 daily gm. respectively of sulfapyrazine without re-appearance of symptoms.

Grateful appreciation is due Dr. Warren Cox of Mead Johnson & Co. for the generous supply of sulfapyrazine used in this study. Sgt. William Wolf rendered invaluable aid in administrative problems.

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Summary and Conclusions. 1. Daily doses were administered over a 30 day period to 2 comparable military groups—1 gm. daily of sulfapyrazine to 1 group, 1 gm. of sulfadiazine to the other.

2. Sulfapyrazine and sulfadiazine were both effective in decreasing streptococcal and non-streptococcal respiratory disease rates.

3. Some indication was obtained that sulfapyrazine prophylaxis was slightly less toxic than sulfadiazine prophylaxis.

VITAMIN LEVELS IN PERNICIOUS ANEMIA*

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As yet, few quantitative studies concerning the presence or absence of vitamin deficiencies in pernicious anemia have been reported. In the past few years, laboratory procedures have been devised for the measurement of vitamin levels in the blood and urine and are now fairly well standardized. Vitamins A and C and carotene can be determined with reasonable accuracy on blood plasma. The B complex vitamins are measured in the urine by chemical and biologic assay, before and after the administration of a test dose. From previously reported studies,¹ there appears to be a definite relationship between the early clinical manifestations of a B complex deficiency and the vitamin levels as determined in the laboratory. The application of these new procedures to patients having pernicious anemia should afford a more exact method of study of this disease and enable one to determine the presence or absence of vitamin depletion in such patients.

Material. Eight patients having pernicious anemia were studied, 7 of whom had the characteristic hematologic picture, as well as glossitis, papillary atrophy of the tongue, and early neurologic changes. The 8th patient was in remission at the time of this study. Two had a diarrhea of from 10 to 15 stools per day. The oldest patient was 65; the youngest, 37. There was an equal number of males and females. No evidence of other organic or debilitating disease was demonstrable.

For the purpose of comparison, 2 other groups were studied: (1) 25 patients diag-

nosed clinically as having a B complex deficiency; and (2) 30 medical students who were used as normal controls.

Method. The method of study was similar to that described in a previous communication.¹ All patients were hospitalized and given a standard diet adequate in protein and calories, but low in vitamins. The approximate composition of the diet was: protein, 60 gm.; fat, 100 gm.; carbohydrate, 325 gm.; total calories, 2500. The estimated vitamin content of this diet was: vitamin A, 130 international units; vitamin C, less than 10 mg.; thiamin, 300 gamma; riboflavin, 250 gamma; nicotinic acid, less than 5 mg.

Vitamin C was measured in the Evelyn photoelectric colorimeter using Tillman's dye and the technique of Mindlin and Butler.² Vitamin A determinations were made by the method of Kimble.³ The vitamins of the B complex were determined in the urine before and after the following test doses: thiamin, 1 mg. intramuscularly; riboflavin, 5 mg. orally; nicotinic acid amide, 500 mg. orally; pyridoxine, 50 mg. orally. Thiamin was first determined by the yeast fermentation technique of Atkins, Schultz, and Frey,⁴ and later by a modification of the thiochrome method. Riboflavin was measured by direct fluorometric method of Ferrebee,⁵ omitting the adsorption and elution steps. The nicotinic acid load test was done as described by Perlzweig, Sarett and Margolis.⁶ F₂ values were determined fluorometrically and paralleled those for nicotinic acid excretion. Pyridoxine was determined colorimetrically by the technique of Scudi *et al.*⁷

Results. VITAMIN A. None of the group studied had any of the findings usually

* This study was supported by a grant from the John and Mary R. Markle Foundation and the Anna H. Haues Research Fund.

attributed to a vitamin A deficiency. Although the plasma levels of A in the deficiency group and in the patients having pernicious anemia were below those of the normal controls, still the means of

these 2 groups were above the suggested lower limit of normal and were essentially the same (Fig. 1). Although the carotene values in the pernicious anemia patients were lower than those of the

TABLE 1.—ARITHMETICAL MEANS OF VITAMIN LEVELS

Vitamins*	Student controls	B complex deficiencies	Pernicious anemia patients	Suggested lower limit of normal
A	114	89	85	75
Carotene	265	205	154	165
C	0.67	0.33	0.42	0.15
Nicotinic acid	88	48	60	65
Thiamin (control period)	668	454	529	400
Thiamin (load test)	422	152	203	300
Riboflavin (control period)	810	387	399	400
Riboflavin (load test)	3333	2127	2375	2200
Pyridoxine	7	5	6.5	4.7

* Vitamin A—International Units per 100 cc. of plasma.

Carotene—International Units per 100 cc. of plasma.

Vitamin C—Mg. per 100 cc. of plasma.

Nicotinic acid—Urinary excretion in mg. in 14 hours after 500 mg. oral test dose.

Thiamin—Control period—Urinary excretion in gamma in 24 hours.

Load test—Urinary excretion in gamma in 24 hours after 1 mg. intramuscularly.

Riboflavin—Control period—Urinary excretion in gamma in 24 hours.

Load test—Urinary excretion in gamma in 24 hours after 5 mg. orally.

Pyridoxine—Urinary excretion in mg. in 4 hours after 50 mg. orally.

LEVELS OF VITAMIN A IN BLOOD PLASMA

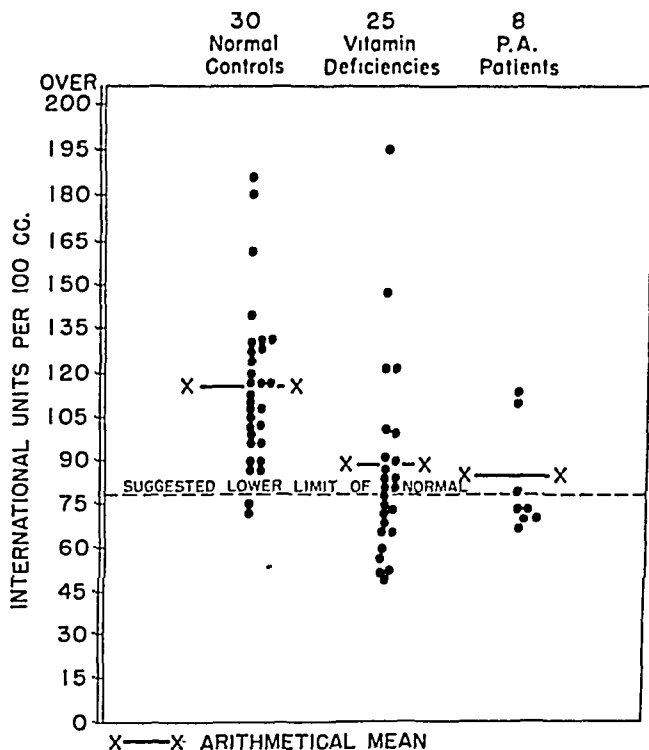


Fig. 1

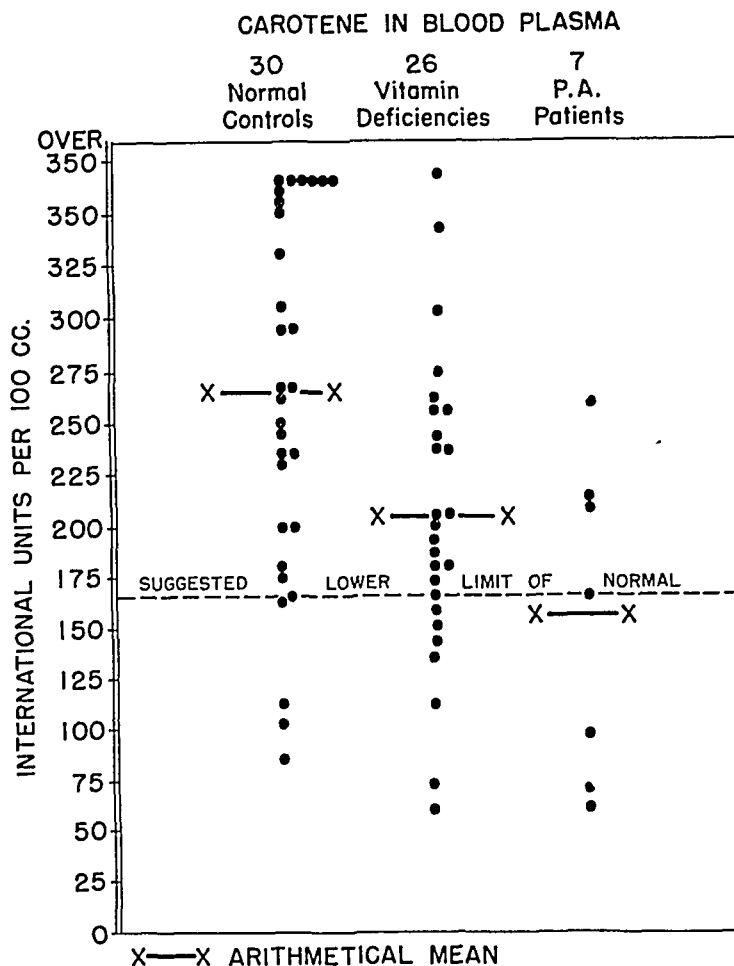


FIG. 2

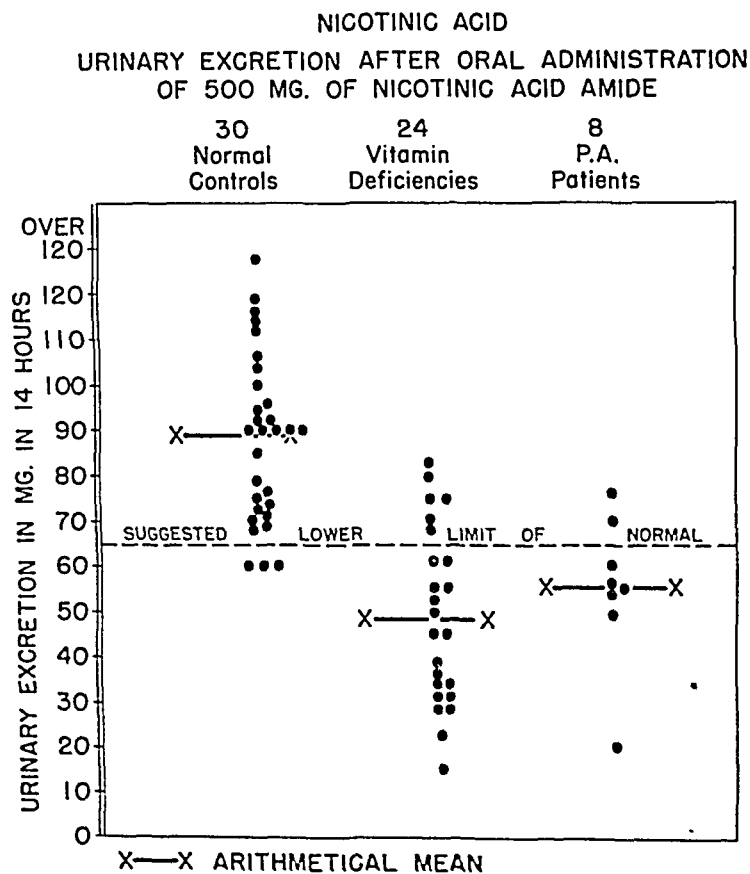


FIG. 3

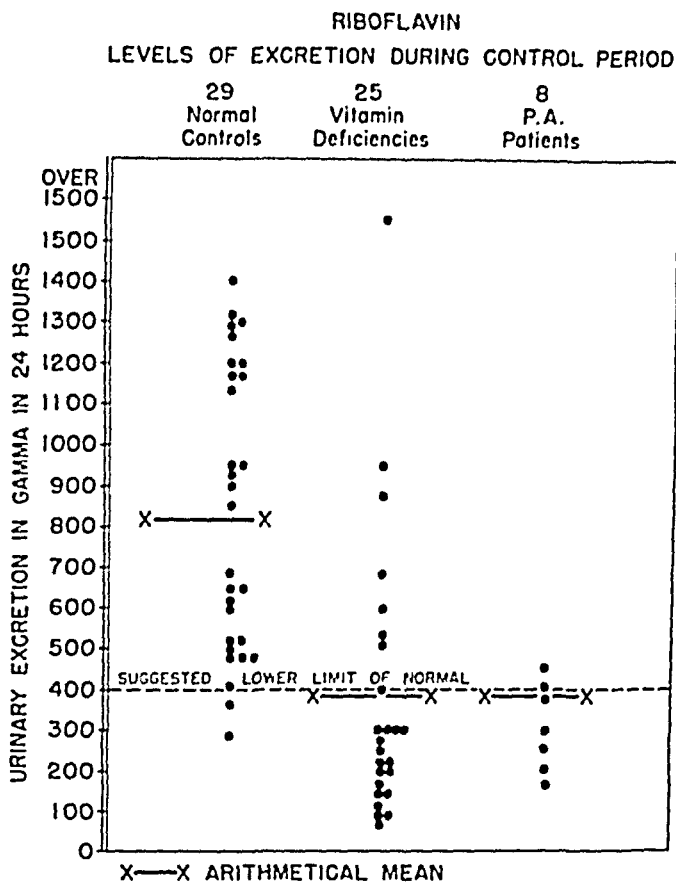


FIG. 4

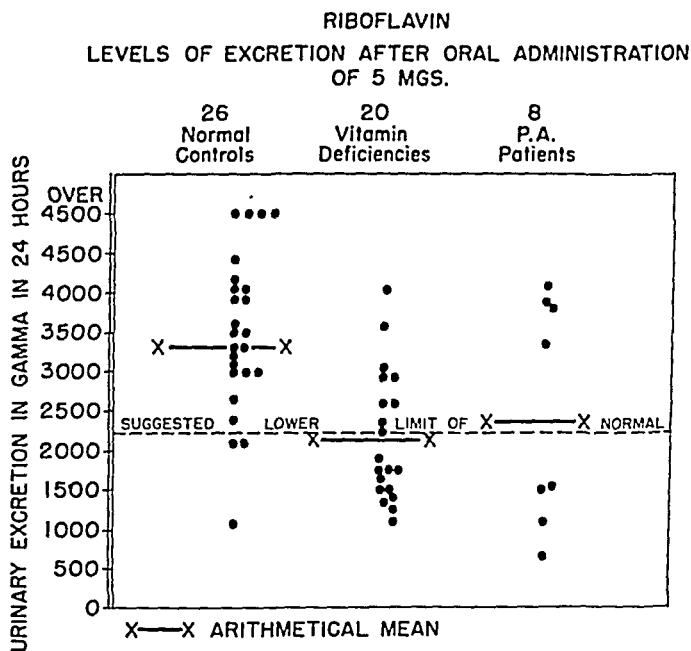


FIG. 5

deficiency group, still the difference was not statistically significant (Fig. 2).

VITAMIN C. There were individuals in each group whose plasma contained no measurable vitamin C. A comparison of the distribution between groups, however, showed no marked difference.

NICOTINIC ACID. A comparison of the urinary excretion of nicotinic acid in the 3 groups, following the administration of 500 mg., is shown in Figure 3. It will be noted that the mean of the patients having pernicious anemia was essentially the same as that of the deficiency group, but

dose, the thiamin levels in the pernicious anemia patients were practically the same as those in the deficiency group, the distribution in general following that of riboflavin. Again, both fell well below the normal control values (Fig. 6).

PYRIDOXINE. There were no significant differences in the distribution of values of pyridoxine excretion between normal individuals and those with pernicious anemia.

Discussion. It is generally recognized that the glossitis, cheilosis, papillary atrophy of the tongue, and parasthenia of the extremities which one sees in patients

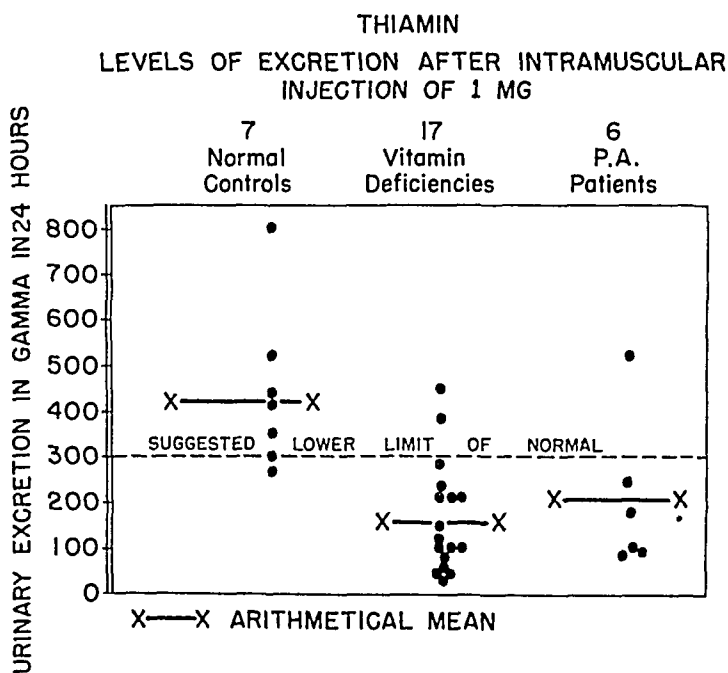


FIG. 6

both were considerably lower than that of the normal controls. Two-thirds of the patients with pernicious anemia, as well as those having a B complex deficiency, had values below the suggested lower limit of normal.

RIBOFLAVIN. The distribution of values for riboflavin excretion before and after the test dose is shown in Figures 4 and 5. The pernicious anemia group had values which were practically the same as those of the patients having a B complex deficiency. Both were significantly lower than those of the normal controls.

THIAMIN. Before and after the test

having pernicious anemia and sprue are frequently indistinguishable from those same findings when they occur in patients having a frank B complex deficiency. It has been suggested⁸ that a deficiency of the B complex is common in both pernicious anemia and sprue and often explains many of the findings which are observed in these diseases. It is well known that there is no constant relationship between the degree of anemia and the severity of the above-mentioned symptoms. Although the administration of the B complex vitamins has no hematologic effect, neurologic lesions in experimental animals can be

prevented by the use of these substances.⁹ The favorable influence of nicotinic acid on glossitis without alteration of the blood picture also has been noted.¹⁰ Hansen¹¹ reports a case of a 39 year old white male with sprue who responded satisfactorily to intramuscular liver therapy. The patient later developed classical signs of pellagra while receiving the liver extract. These subsided promptly after the administration of nicotinic acid, supporting the belief that refined liver preparations may not contain adequate amounts of pellagra-preventive substances.

The present study was undertaken in an effort to determine the possible co-existence of an underlying B complex deficiency in patients having pernicious anemia. A study of the table and charts shows how closely the vitamin levels in the group having a B complex deficiency parallel those of the pernicious anemia patients. There was no apparent correlation between the degree of anemia and the vitamin levels in either group of patients. As might be expected, the levels of vitamin A and carotene in general reflected the nutrition of the group; and although lower than those of controls, were within normal limits. This is in striking con-

trast to studies on sprue, previously reported, in which a profound depletion of vitamin A and carotene in the plasma was found.¹² The blood levels of vitamin C were essentially normal.

Although the group studied is small, the definite lowering of nicotinic acid, thiamin, and riboflavin levels in these patients cannot be overlooked. One is impressed by the similarity of the distribution of the values in the pernicious anemia group to those in the vitamin deficiency patients. Therefore, the occasional reports of improvement of oral lesions on B complex therapy alone is not surprising. Complete recovery after liver therapy may be explained by the presence of sufficient accessory factors in the preparation used as well as by the well-known stimulating effect of liver upon the appetite with a resulting increase in dietary intake. The results of this study would justify the administration of B complex vitamins to patients having pernicious anemia if liver extract alone fails to produce a satisfactory response.

Conclusion. The laboratory determinations of vitamin levels of 8 patients having pernicious anemia show a coexisting deficiency of the B complex vitamins.

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THE USE OF A HISTAMINE ANTAGONIST, BETA-DIMETHYLAMINETHYL BENZHYDRYL ETHER HYDROCHLORIDE, IN ALLERGIC DISEASE*

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CONSIDERABLE evidence has accumulated in recent years emphasizing the rôle of histamine in anaphylactic and allergic reactions. In a review of the subject, Dragstedt³ points out that while histamine may not account for all the manifestations of anaphylaxis, its relation to the reaction is given strong support by several established facts. It may mimic many of the important anaphylactic reactions; it is present in the tissues where such reactions take place; and its release in association with anaphylaxis has been conclusively demonstrated. In the field of human hypersensitivity the evidence involving histamine has been less conclusive. The similarity between the results of histamine injection and cold allergy was noted by Horton, Brown and Roth.⁵ Rose and Browne¹⁰ found that the histamine content of the blood of normal individuals showed great fluctuations in contrast to relatively constant levels in normal subjects. Elevated blood histamine in allergic diseases has been reported by some investigators,¹ while others⁹ have failed to find any significant correlation between allergy and histamine content of the blood. Katz⁶ has recently shown that histamine is released from human skin on contact of allergens in sensitive individuals. The search for a substance that counteracts histamine has therefore offered a hopeful approach to the non-specific treatment of allergic disease. Recently a group of synthetic benzhydryl alkaline ethers have been investigated and found to be markedly effective in preventing histamine and anaphylactic shock in guinea pigs.^{7,8} The most effective member of this group is beta-dimethylaminethyl benzhydryl ether hydrochloride. Its marked histamine antagonism, inhibition of anaphylaxis, and relatively low toxicity have suggested

its trial in the allergic conditions commonly encountered in everyday practice.

A group of 47 patients with a variety of allergic complaints including asthma, seasonal hay fever, urticaria, angioneurotic edema, eczema and allergic headache, were given the drug orally in doses of 50 to 600 mg. daily for varying periods of time.

The patients in the series were subjected to complete allergic studies, including skin tests. They were to some degree selected in that the drug was administered in instances where the specific etiology could not be determined; in cases where specific treatment had not advanced far enough to become effective in preventing symptoms; or where the usual measures at our disposal had failed to produce the desired results.

Urticaria and Angioneurotic Edema. Urticaria and angioneurotic edema were studied; 2 were of the acute variety, while 11 had chronic or recurrent symptoms of 1 month to 30 years duration, permitting a more accurate evaluation of the drug. Acute urticaria tends to improve spontaneously, and any results obtained in such cases must be judged in this light. All patients in the present study received some degree of improvement, ranging from the relief of pruritus to complete disappearance of lesions shortly after the first dose of 50 mg. The effect of the drug was noted within 1 hour and lasted in most instances from 2 to 12 hours, with a gradual return of symptoms when discontinued. Curtis and Owens² also reported improvement in a series of 18 cases of urticaria with recurrence of lesions upon discontinuance. Dosage in our study was found to be an individual problem. From 50 to 200 mg. daily was required to maintain results in the majority. In 1 case,

* The drug (Benadryl) for this investigation was supplied by Parke Davis & Co

however, 600 mg. was necessary to insure comfort and complete relief of symptoms. An effort was made to determine the etiologic factors in each case, and the drug was frequently discontinued to learn the effect of trial diets. When symptoms recurred, or when they were reproduced by an offending food, the drug was able to control the urticaria promptly. In this manner, considerable information regarding the etiology of the urticaria was obtained with a minimum of discomfort to the patient. Where the cause of the urticaria could not be determined, the minimum amount of the substance necessary to control symptoms was ascertained. A tendency to develop tolerance was noted when administered over long periods of time. As a palliative measure in these conditions, it appears to be the most effective agent yet encountered. It should

He was then placed on the experimental drug in doses of 50 mg. every 2 hours. The urticaria and pruritus subsided following the initial dose, and within 24 hours the edema and all lesions had completely vanished. No change was noted in the polyarthrititis. An attempt was made to reduce the dosage after a few days, but when given less often than 3 times daily (150 mg.) the urticaria recurred. This was immediately controlled with a single dose of 50 mg. At the time of this paper the patient still required 150 mg. daily.

Atopic Eczema. The effect of the drug on 4 patients with atopic eczema of long duration was observed. The most significant occurrence was a marked reduction of pruritus in 2 of these cases. This was accomplished with very small doses. No definite effect on the lesions themselves were noted, even when larger amounts

TABLE 1.—CASES STUDIED

Diagnosis	No.
Urticaria and angioneurotic edema . . .	13
Eczema (atopic)	4
Asthma (non-seasonal)	9
Hay fever (ragweed)	19
Allergic headache	2
Total	47

not, however, be used to the exclusion of allergic investigation necessary to determine the underlying factors of each case. For the patient to continue to remain well, it is extremely important to determine the causative factors and thus eliminate the offenders.

Two cases (E. M. and H. L.) received only partial relief from symptoms when on either the diet or the drug. Symptoms were much less on dietary management, but on combined treatment, complete relief was obtained. Patient R. D., a 58 year old male, developed urticaria, polyarthrititis and a severe generalized edema of the entire body after administration of 350,000 units of penicillin for a corneal ulcer. During the 1st week of symptoms, general medical measures, including ephedrine, adrenalin and large doses of vitamin C, were instituted, without results.

were used. In 1 patient (D. D.) the lesions were thought to improve when the drug was first administered. Later it was found that symptoms fluctuated with the ingestion of certain foods. Good results have since been obtained by dietary measures alone. Another case (B. H.) had asthma in addition to eczema, but no effect on the former was noted during the course of treatment. The chief attribute of the drug in such cases seems to be its effect on pruritus.

Asthma. No definite beneficial effect could be attributed to the drug in the 9 cases of asthma under consideration. In patient T. T., female, the condition was recurrent and there were definite psychogenic elements involved. Early in treatment she claimed relief for 3 hours after taking 50 mg. at bedtime. Later, however, she received no benefit whatsoever

TABLE 2.—URTICARIA AND ANGIONEUROTIC EDEMA

Beta-dimethylaminethyl benzhydryl ether hydrochloride

Case	Age	Sex	Duration and type	Other allergy	Daily dose (mg.)	Start	End	Side-effects	Results	Remarks
B. G.	41	F	3 mos. Chronic	..	50-150	3/30	5/11	Dizziness; GI upset	Improved	Small doses did not help; definite impr. with large doses; unable to cont. because of side-effects
J. G.	35	F	30 yrs. Recurrent	VR	50	4/27	5/4	.. .	Improved	Immed. response; remained symp.-free on diet after stopping drug; compl. control by elim. of food offenders
L. G.	50	M	4 mos. Chronic	..	50-200	5/4	5/23	.	Itching relieved; no effect on urticaria	
M. K.	20	M	1 yr. Recurrent	M and HF	150-50	6/27	7/3	Improved	Compl. relief after 1 dose; symp. did not recur after drug was elim.
M. K.	42	M	1 mo. Chronic	HF	150-50	4/2	4/14	Weakness	Improved	Immed. impr. on 150 mg.; reduced to 50 mg. after 5 days; aggravation from offending foods when on low dosage; milk main offender
R. B.	27	F	10 days Acute	..	50-100	2/16	4/14	Drowsiness	Improved	Relief of symp. 30 min. after dose; lasting 10 to 12 hours; recurrence of symp. several times when drug removed
W. P.	57	M	5 yrs. Recurrent	VR	100	5/14	6/30	. .	Improved	Relief in 30 min. on 100 mg. p.r.n.; also noted some impr. in nasal symp. on taking 1 in A.M.; aspirin sensitivity later revealed as cause
M. A.	23	F	6 mos. Chronic	HF	50-150	1/23	4/3	. .	Improved	Immed. relief on drug; good on smaller dose, but symp. recurred if discontinued; now takes 1 p.r.n.; food and emotions have definite influence
E. M.	28	F	2 yrs. Chronic	..	50	2/7	7/25	Improved	Incomplete relief on diet; Mechanical factors also play a rôle; complete relief from 50 mg. when on diet; both are necessary
R. W.	13	M	4 yrs. Recurrent	..	50	7/13	7/27		Improved	Definite impr. on 50 mg., but incomplete until food offenders eliminated; now no hives without drug, when on diet
H. L.	42	F	2 yrs. Recurrent	.	50	7/30	8/11	Fatigue	Improved	Less urticaria with drug; complete relief when used with food elim.
R. D.	58	M	2 wks. Acute	..	150-600	8/4	8/18	.	Improved	Urticaria and severe generalized edema after penicillin; urticaria subsided soon after initial dose; edema was gone in 24 hours; patient had to be maintained on 150 mg. to remain symptom-free; polyarthritis developed when urticaria impr. and not benefited by drug
A. P.	42	F	6 wks. Chronic	..	150	7/27	8/18	Drowsy	Improved	Immed. relief with drug; recurrence when discon.; impr. with food elim.

VR—vasomotor rhinitis, HF—hay fever M—migraine.

from the same or increased dosage. Her improvement coincided with the alleviation of emotional stress incident to family affairs. Another case, A. V., started with 50 mg. doses 3 times daily showing no improvement until 400 mg. were reached, but remained well after the drug was discontinued. Previous episodes in this patient seemed to run a similar course without any symptomatic medication. It

is therefore doubtful whether there occurred any real therapeutic effect in any of these cases.

Hay Fever. Nineteen cases of ragweed hay fever were treated with the drug during the 1944 ragweed season. Among the patients in this series were some who had received little or no pollen treatment, and others who despite pollen therapy still had sufficient symptoms to justify other

TABLE 3.—ATOPIC ECZEMA

Case	Age	Sex	Duration	Other allergy	Etiology	Beta-dimethylaminethyl benzhydrol ether hydrochloride				Results	Remarks
						Daily dose (mg.)	Start	End	Side-effects		
L. B.	46	F	2 yrs.	..	P, M	50-200	5/26	6/15	Dizziness	0	Symptoms from spring to cold weather; some relief from desensitization to pollens and molds
D. D.	14	M	13 yrs.	HF and A	F	50-150	5/12	6/19	0	0	Impr. at first, but later had no effect
B. H.	29	F	13 yrs.	A	D, F	50-150	6/6	6/16	0	Relief from pruritus	Developed attack of asthma during course of drug
G. S.	39	F	4 yrs.	..	F	50-150	3/30	4/27	0	Relief from pruritus	No change in skin condition

P—pollen. M—molds. D—dust. F—food.

TABLE 4.—ASTHMA

Case	Age	Sex	Duration	Other allergy	Skin tests	Beta-dimethylaminethyl benzhydrol ether hydrochloride				Results	Remarks
						Daily dose (mg.)	Start	End	Side-effects		
H. B.	46	M	7 yrs. Recur.	U and HF	E, P, F	50-100	5/15	5/30	0	0	Impr. after sufficient spec. desensitization
H. B.	49	F	17 yrs. Recur.	VR and GI	D, E, F, P	50	6/1	6/4	Weakness	0	Given for acute episode; since, controlled by diet and desensitiz.
E. F.	21	F	15 yrs. Chr.	GI and VR	F, P	50	6/2	..	Faintness	...	Unable to continue because of side-effects
H. G.	59	M	2 yrs. Recur.	...	F, D	50	5/15	6/5	0	0	Given for acute exacerbation
F. K.	51	M	12 yrs. Recur.	...	P, D, E	150	5/18	6/4	0	0	Given for acute exacerbation
T. P.	50	F	10 yrs. Chr.	...	F, D	50	5/21	7/6	0	?	Given at bedtime when sympt. are usually present; felt that it helped at first, but later no effect
L. S.	15	F	1 yr. Recur.	HF	P, M	150	8/23	9/5	0	0	
R. W.	55	F	20 yrs. Chr.	HF	D, P, M	150	9/21	9/25	Weakness Drowsiness	0	
P. V.	20	F	3 yrs. Recur.	VR	F, D, P, M	100-400	7/25	8/1	0	Quest. impr.	Started on 100 mg. daily, incr. to 100 mg. 4 times daily; on 4th day remained well when drug was discontinued; patient morose away; no follow-up

U—urticaria. HF—hay fever. VR—vasomotor rhinitis. GI—gastro-intestinal allergy. A—asthma.
E—epidermal. P—pollens. D—dust. F—foods. M—molds.

measures. Symptoms were correlated with the daily pollen count, atmospheric conditions, and comparison with an adequate number of control patients in order to properly evaluate the effect of the drug. As pointed out in a recent hay fever study,⁴ failure to employ these considerations have led to erroneous conclusions,

TABLE 5.—HAY FEVER

Case D. D.	Age 28	Sex M	Dura- tion 6 yrs.	Status of pollen treat- ment 1944 I	Beta-dimethylaminethyl benzhydryl ether hydrochloride					Results Rhinitis; sl. impr. for short time	Remarks Symp. continued to fluctuate with pollen count
					Daily dose (mg.) 150	Start 8/23	End 9/1	Side-effects Dizziness			
G. B.	65	F	13 yrs.	I	150	8/29	9/8	GI upset	0	Symp. fluctuated with pol- len count	
S. F.	35	F	7 yrs.	I	150	8/23	9/5	0	0		
M. F.	31	F	2 yrs.	I	150	8/28	9/20	0	0	Had asthma all through sea- son; also mold sensitive	
S. G.	12	F	5 yrs.	I	150	8/28	9/12	Dizziness	0	Food sensitivity present	
S. G.	25	M	10 yrs.	I	150	9/11	9/19	0	0		
E. H.	46	F	8 yrs.	I	150	8/30	9/11	GI upset	0		
A. K.	30	M	15 yrs.	I	150	8/22	8/29	0	0		
M. L.	12	F	3 yrs.	I	50	9/6	9/13	0	0	Symp. alleviated when pol- len count dropped	
H. R.	33	M	10 yrs.	A	150	8/30	9/6	Fatigue	0	Symptoms fluctuated with pollen count and weather conditions; dust sensitive	
F. S.	6	F	5 yrs.	A	150	8/11	9/2	0	0		
D. S.	15	F	4 yrs.	I	150	9/1	9/8	0	0		
S. S.	10	F	3 yrs.	A	150	9/8	9/15	Drowsiness	0		
A. V.	26	F	6 yrs.	I	150	8/30	9/13	0	0		
B. W.	23	F	6 yrs.	I	150	8/30	9/8	G.I. upset, drowsiness	±	Relief of symptoms day after drug started; remained good, but was forced to discontinue drug because of side effects; hay fever was minimal remainder of season without drug	
W. T.	34	M	10 yrs.		150	8/30	9/8	0	±	Continued having symptoms but to a lesser degree; pollen count was dropping, no symptoms after drug was discontinued	
P. H.	28	F	10 yrs.	N	50 50	5/18 7/2	5/21 7/7	0 0	0 0		
L. K.	36	M	3 yrs.	N	150	9/6	9/9	0	0		
R. S.	32	M	10 yrs.	N	150	9/6	9/30	0	Sl. impr. of rhinitis	Symp. fluctuated with pol- len count	

A—adequate. I—inadequate. N—none.

TABLE 6.—ALLERGIC HEADACHES

Beta-dimethylaminethyl benzhydryl
ether hydrochloride

Case	Age	Sex	Duration	Other allergy	Daily dose (mg.)	Start	End	Side-effects	Results	Remarks
J. E.	43	M	1 yr.	VR	50	6/2	6/8	0	0	Has cont. headaches; gets definite relief from intradermal histamine injections
A. P.	27	F	15 yrs. Recur.	...	50	5/18	5/25	0	0	Well controlled for most part with dietary régime; tried drug when she went off diet; used on 4 occa.; 3 times no relief; once questionable

VR—vasomotor rhinitis.

as to the efficacy of numerous methods for the treatment of hay fever. Two patients received 50 mg. daily, while 17 were given 150 mg. daily in 3 divided doses. In some instances, improvement was observed which coincided with drops in the pollen count, and similarly observed in control subjects. Two patients (B. W. and W. T.) reported definite improvement shortly after the drug was started. Treatment was discontinued after a period of 8 days in both cases to determine whether symptoms would recur. In neither did symptoms appear during the remainder of the season. This occurred, however, during a decreasing pollen content of the air, making any definite evaluation of results in these 2 instances impossible. Patients B. D. and R. S. reported slight, but temporary improvement of their rhinitis, but one could not tolerate the drug. Both patients received similar relief of symptoms upon ingestion of $\frac{1}{16}$ gr. doses of atropine. The remaining 15 patients failed to note any benefit that could be directly attributed to the drug.

Allergic Headache. The drug was tried in 2 cases of headache which were thought to be of allergic origin. Neither case obtained relief when given for acute headache. Both have subsequently been controlled by dietary measures.

Toxicity. Of 47 patients in this series, 16 complained of some side-effects. The most common symptoms attributed to the medication were drowsiness, dizziness, weakness, faintness, fatigue and gastrointestinal upsets. In 3 patients it was found necessary to discontinue the drug because of the side-effects. In a few other cases dosage was necessarily restricted by these annoying symptoms. The majority, however, were able to tolerate the medication without undue reactions.

Comment. Beta - dimethylaminethyl benzhydryl ether hydrochloride appears to be an extremely effective agent in the palliative treatment of urticaria and angioneurotic edema. Its effect is rather short,

and symptoms in chronic cases tend to recur after it is discontinued. In the cases under study in this report, it served as an excellent aid in controlling discomfort while the necessary procedure for the determination of etiologic factors were carried out. Its use in chronic cases over long periods of time without attention to possible underlying allergic etiology does not seem justified, especially in view of occasional side-effects, and increased tolerance on extended use.

The drug also has some effect in reducing the pruritus incident to atopic dermatitis. It may likewise be effective in pruritic conditions of other origin.

Experimental evidence in guinea pigs indicated that this substance is one of the most potent histamine antagonists yet found. The good results observed in urticaria, and the failure to benefit other atopic conditions, suggest the possibility that histamine may play a greater rôle in urticaria than in asthma, hay fever, and other allergic manifestations. There is also the possibility that the antihistamine substance may be necessary in larger amounts to produce effects in conditions other than urticaria. Routes of administration other than oral may perhaps be necessary to produce the desired results in other allergic conditions.

Summary. 1. Beta-dimethylaminethyl benzhydryl ether hydrochloride, a potent histamine antagonist in guinea pigs, was administered to 47 patients with various allergic complaints.

2. Marked benefit, which continued in most cases as long as the drug was administered, was noted in urticaria. Relief of pruritus was noted in some cases of atopic eczema. No benefit was apparent from its use in asthma, hay fever, or allergic headache.

3. The results suggest that histamine may be a more important factor in urticaria than in other allergic conditions.

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CAPILLARY MICROSCOPY

WITH SPECIAL REFERENCE TO CAPILLARY PETECHIÆ

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THE actual observation of nail-bed capillaries by microscopy (capillaroscopy) is disarmingly easy. The technique has often been described. The key to the literature and basic work will be found in the works of Mueller^{4,5} (1922, 1937 and 1939), and in the papers of Wright and Duryee⁶ (1933), Leader³ (1932) and Calander¹ (1940). A special capillary microscope is not essential. Excellent observation is possible with any good laboratory microscope, although a binocular instrument is preferable. Using a standard binocular microscope I have examined the finger nail-beds of over 1000 patients (including many out-patients), without any special preparation and without scrubbing the nails. I observed at least 2 fingers, but usually 3 fingers, and often 4 fingers, of each hand. The patients' ages ranged from 5 to 87 years. The capillary pattern in any particular healthy adult is usually strikingly constant—for example, my own has not varied significantly in over 6 years of almost weekly observation. Nevertheless there is often much variation between the capillaries in a single nail-bed, and sometimes great variation from finger to finger, and between the fingers of both hands. Yet unwarranted inferences have been drawn from variations well within normal. Various specialists have examined the nail-beds of a few dozen patients suffering from a particular disease, and arrived at conclusions without adequate controls in normal persons and patients with other diseases.

Capillary Petechiæ. I first became interested in capillaroscopy as an aid in the study of purpura.² Observations showed that capillary petechiæ were common. Thus there were 100 patients with capil-

lary petechiæ in 533 consecutive patients whose nail-beds were examined microscopically. This incidence (18.4%) is unduly high, because on a basis of previous experience, patients likely to show abnormalities and petechiæ were specially selected. Nevertheless, most types of patient using the medical division of a general hospital were examined contemporaneously, and healthy persons in addition. It is possible to give a general picture of the incidence of these petechiæ.

The patients showing capillary petechiæ have been divided into 3 groups (see table). Group 1 comprises a mixed collection of various types of cases, in each of which type capillary petechiæ were seen once only, or not at all. Among 262 such cases, which included various diseases of the heart, lung, kidney, nervous system, blood and skin, petechiæ were seen 23 times (an incidence of 8.8%). In Group 2 are included diseases in some of which the frequency of capillary petechiæ may prove significant, but where much more evidence is wanted. Group 3 comprises the conditions in which petechiæ were most frequent. They were scurvy, bronchiectasis, arteriosclerosis, purpura simplex, Raynaud's disease and clubbed fingers. Separate figures for clubbed fingers are not given in the table because all patients with clubbing were listed according to their disease. Thus of the 6 patients with bronchiectasis and capillary petechiæ, 5 had clubbed fingers, as had both patients with Fallot's tetralogy, and 2 of the 3 patients with syphilis. Of the 533 patients considered, 23 had clubbed fingers, and 11 of these had capillary petechiæ.

Morphology. The appearance of the petechiæ is shown in the figures, in about

the order of frequency of the various types. No type of petechia was pathognomonic of any disease. Petechiæ often arose from abnormal congested capillaries with sluggishness or stasis of blood flow. Petechiæ seemed to arise nearly always from the loop joining the arterial and venous limbs of the capillary, and very rarely from these limbs. Occasionally, petechiæ were seen actually separating from the capillary. In 2 patients, after such separation, the capillary re-attached itself to the petechia, and then the petechia again became detached. Once

neously. In others, skin petechiæ precede or follow the findings of capillary petechiæ. But many patients show capillary petechiæ when no skin petechiæ were seen at any time. (Compare the similar discrepancy when retinal or mucous membrane hemorrhages are present and skin hemorrhages absent, or when visceral petechiæ are seen at necropsy without skin petechiæ.) Occasionally the skin has presented a widespread massive petechial eruption, but petechiæ were absent at the nail-bed.

In spite of these tantalizing discrepancies, it is clear that capillary petechiæ are

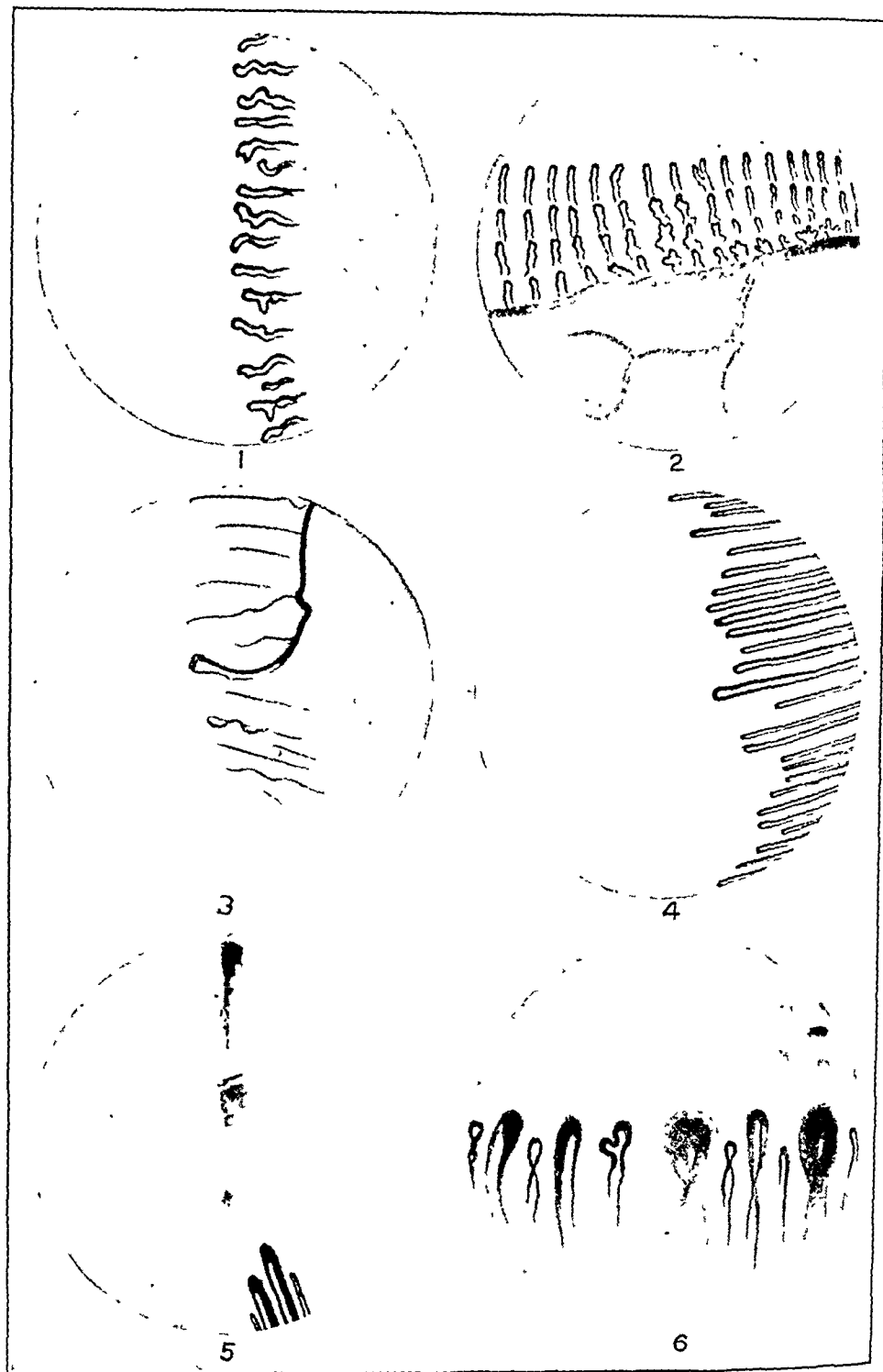
ANALYSIS OF 100 PATIENTS WITH CAPILLARY PETECHIÆ FOUND AMONG 533 CONSECUTIVE PATIENTS RECENTLY EXAMINED BY CAPILLARY MICROSCOPY

Group	Disease	No. cases examined	No with capillary petechiæ
1	Miscellaneous (see text)	262	23 (8.8%)
2	Rheumatic fever (acute or chronic)	54	9
	Diabetes mellitus	26	4
	Epilepsy (idiopathic)	22	3
	Syphilis	13	3
	Schönlein-Henoch purpura	11	3
	Pulmonary tuberculosis	10	3
	Osteo-arthritis	8	2 (23.1%)
	Infective polyarthritis	5	2
	Gout	4	2
	Virus hepatitis	7	2
	Malaria	4	2
	Pituitary diseases	3	2
	Fallot's tetralogy	2	2
3	Arteriosclerosis	39	11
	Purpura simplex	31	10
	Scurvy	11	7 (27.4%)
	Bronchiectasis	14	6
	Raynaud's disease	7	4
	Clubbed fingers (see text)		
	Total	533	100 (18.4%)

I saw the loop of a capillary (grossly distended through stasis of blood) become detached from the limbs to form a hemorrhage, and on several occasions a portion of such a loop became detached. Occasionally the petechia was shaped like a capillary cast. Petechiæ when first shed were obvious collections of red blood cells, later they became darker, and appeared as a rather homogeneous mass of pigment. If examined daily, the petechiæ moved further and further away from the capillaries, and faded.

Discussion. In some patients capillary petechiæ and skin petechiæ occur simulta-

neously, particularly in scurvy, bronchiectasis and other diseases with clubbed fingers, arteriosclerosis, purpura simplex and Raynaud's disease. In these conditions, the incidence of capillary petechiæ appears to be specially significant. When the frequency of macroscopic petechiæ in various organs at necropsy is recalled, the incidence of these microscopic petechiæ is less surprising. Capillaries are prone to petechiæ formation in disease. I have not as yet seen capillary petechiæ in the healthy. It is possible that for every petechia visible to the naked eye in any patient, there are many microscopic pete-



KEY TO FIGURES

All capillaries were seen at $\times 60$ and drawn to scale.

Figs. 1 to 4.—Types of normal capillaries.

Figs. 5 to 11.—Types of petechiae.

Fig. 11.—Petechia in formation.

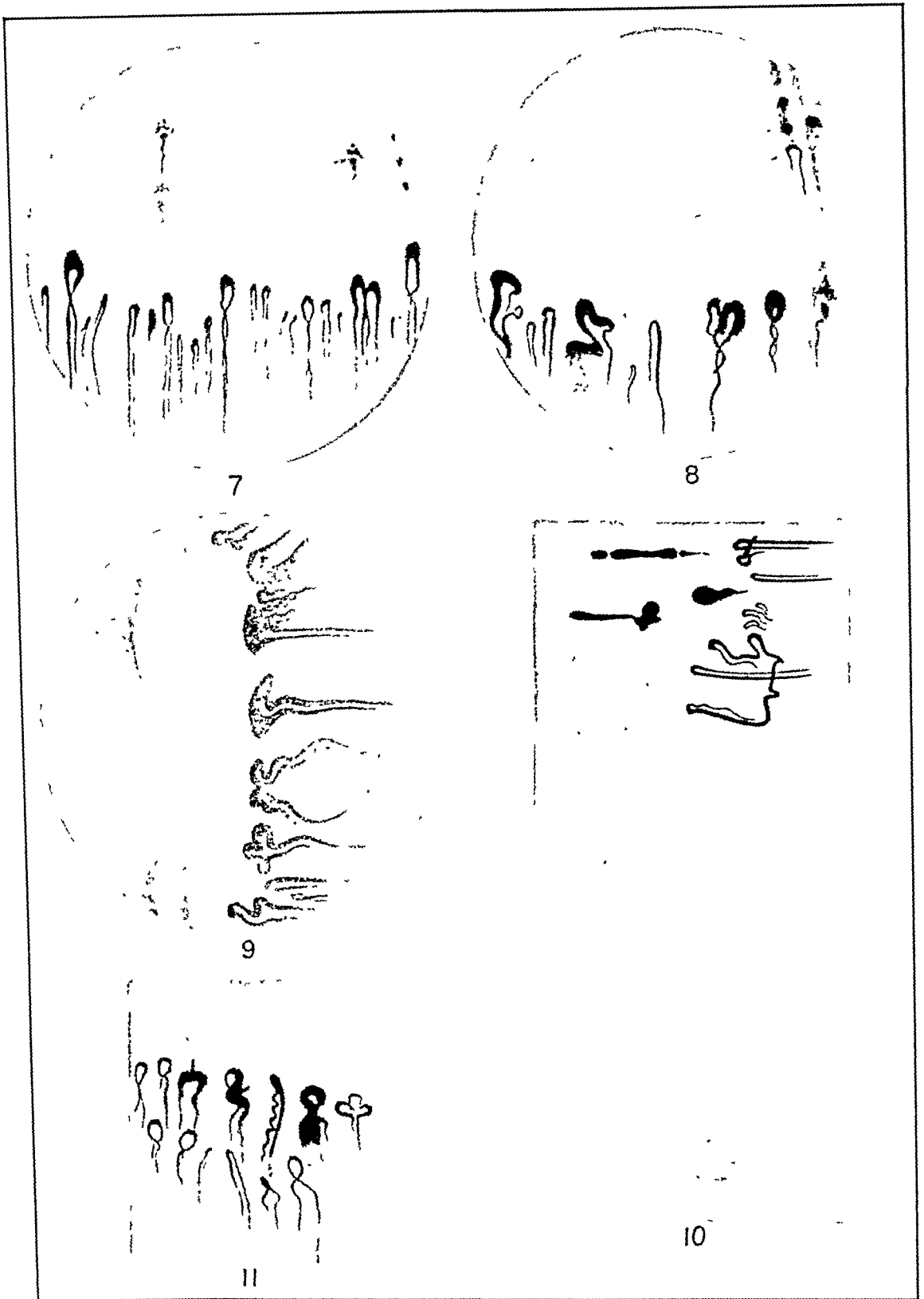


FIG. 5.—Patient with pulmonary tuberculosis.

FIG. 6.—Patient with recurrent skin purpura following influenza.

FIG. 7.—Patient with chronic rheumatic carditis.

FIGS. 8, 9 and 11.—Patients with Raynaud's disease.

FIG. 10.—Patient with Fallot's tetralogy.

chiae formed. Nail-bed capillaroscopy will render some of these visible, but at the nail-beds only a minute fraction of the capillaries of the body can be seen, and it is likely that many petechiae will be formed at other sites. In purpura simplex, only a small fraction of skin surface may show hemorrhage, and most of the skin will be normal. Yet in 1 of 3 cases of purpura simplex, examination (often but once) of the tiny proportion of the body's capillaries at the nail-bed, will reveal petechiae. And the incidence in some diseases is even higher.

Capillaroscopy gives no trouble or pain to the patient, and is gratifying and stimu-

lating to the observer. Here is an additional field for the study of human physiology and pathology.

Summary. There were 100 patients with capillary petechiae in 533 consecutive patients whose nail-beds were examined by capillary microscopy. The conditions in which such petechiae were frequent were arteriosclerosis, purpura simplex, scurvy, bronchiectasis, Raynaud's disease and clubbed fingers. Capillaries are prone to petechiae formation in disease. Many patients showed capillary petechiae when no skin petechiae were present at any time, and occasionally *vice-versa*.

I thank Mr. J. R. M. Whigham for help, Dr. R. Karpin for coöperation, Mr. R. Startup for paintings of capillaries, and Dr. J. E. McCartney and Mr. J. E. Andrews for photographs of these paintings.

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CLINICAL AND LABORATORY STUDIES OF LIVER FUNCTION IN THERAPEUTIC MALARIA*

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THE exact status of the pathologic changes occurring in the liver during malaria infections is not well understood, but the topic has received increasing attention in recent investigations.^{1,3,4,6} Our interest in this subject was aroused when several cases of jaundice occurred as complications of therapeutic malaria at the Neurosyphilis Center of this general hospital. Furthermore, in view of the current emphasis on dietary constituents as a protection against or treatment for hepatitis, it was decided that an excellent opportunity to evaluate the protective qualities of special diets existed.

A critical study to determine the degree of liver involvement during malaria was facilitated by the active utilization of therapeutic infections on the Neurosyphilis Center. The patients were under well-controlled conditions, and the duration of clinical malaria was often considerably longer than the customary period of treatment for neurosyphilis.

Material and Method. The patients included in this study were all soldiers requiring malaria therapy for neurosyphilis. The majority were young men with asymptomatic neurosyphilis, and were otherwise in good general health. Almost every patient had previously received at least 6 months of mapharsen and bismuth chemotherapy prescribed by the Army in the treatment of syphilis. Malaria inoculations were performed by a variety of techniques, including mosquito application, intradermal inocula-

tion, and intravenous inoculations with varying parasite dosages.

The status of the liver was followed with recorded observations, both clinical and laboratory, prior to the onset of malaria paroxysms, periodically during the paroxysms, and following the therapeutic course until the patient's discharge from the hospital.

Clinical observations were made very frequently. During the actual febrile course, the patients were seen at least twice daily by one of the medical officers in attendance. Formal rounds were made daily by at least one of us. Quantitative observations on the following details were made on each patient. General condition: the presence of any digestive symptoms, particularly the condition of the appetite or the presence of nausea and vomiting; the degree of jaundice, if any; the palpability of the liver and spleen; and the presence of edema. Patients' weights were closely followed with observations recorded once or twice weekly.

Complete laboratory data were obtained in each case. Those pertinent to the present investigation were: the hemoglobin; icterus index; van den Bergh reaction, both direct and indirect; plasma proteins with albumin and globulin partition; oral hippuric acid excretion test; the prothrombin time; the cephalin cholesterol flocculation test; and the urinary urobilinogen determination. These laboratory tests were performed before the onset of clinical malaria, and at varying intervals during and after the active febrile course. In the case of those inoculated with vivax malaria, repeat studies were made after the initial 10 paroxysms, and then following each subsequent 5 paroxysms. In those

* All laboratory tests were performed under the direct supervision of DeWitt F. Mullins, Capt., M.C., Chief of Laboratory Service.

† Major Glenn died after this study was completed.

with quartan malaria, the determinations were made after the initial 5 paroxysms, and then after each subsequent 5 paroxysms. In both instances (vivax and quartan), at least 1 follow-up test was performed approximately 10 days after the institution of atabrine therapy. In some cases, 2 and 3 follow-up tests, at different intervals, were performed if the patient was hospitalized long enough.

The patients in the study were divided into 3 groups, designated Groups A, B, and C. There was no selection of patients for this grouping, for they were placed in a consecutive group as they were inoculated. The only exceptions were Negro patients, all of whom were inoculated with quartan malaria. Arbitrary group assignments were here made in order to equally distribute the smaller number of quartan-infected cases. There were 20 patients in each group—15 with vivax malaria and 5 with quartan malaria, making a total of 60 patients in the entire study.

Group A was the control group and received only the routine treatment with no additional medication. They were given the regular hospital diet, which averaged 335 gm. of carbohydrate, 115 gm. of protein, and 164 gm. of fat, for a total of 3300 calories daily. In this group, as well as in the other groups, meals were tolerated best on paroxysm-free days. On febrile days, patients definitely ate less, ingesting very little solid food and mostly liquids. This general digestive status was altered as noted by the specific observations in Table 2.

Patients in Group B were given, in addition to the regular diet as prescribed for Group A, additional protein intake in the form of more high protein foods. They were served with "protein cocktails," 3 times daily, each consisting of a glass of milk containing 1 egg and 1 tbs. of Brewer's Yeast Powder or Aminoids. If 3 cocktails were consumed daily, an extra protein intake of 45 gm. was provided. Group B patients were also given multivitamin capsules, 2, 3 times daily, each containing 2500 units vitamin A, 200 units vitamin D, 1 mg. thiamine chloride, 1.5 mg. riboflavin, 10 mg. nicotinic acid, and 37.5 mg. ascorbic acid. They also received crude liver extract injections (3 cc. each—assayed at 2 reticulogenic units per cc.) intramuscularly every other day.

Group C was on the same regimen as Group B, but received an additional 1000 cc. of intravenous glucose solution daily. This consisted of 5% glucose in saline and 10% glucose in distilled water on alternate days, thus providing each patient in Group C with an added average dose of 75 gm. of glucose intravenously daily during the course of the fever. 100 mg. of ascorbic acid and 50 mg. of thiamine chloride were added to each intravenous infusion. Both Groups B and C were allowed to raise their protein intake as they desired by taking additional Brewer's yeast or aminoids in peanut butter sandwiches, cereal, etc.

Results and Discussion. 1. **CLINICAL OBSERVATIONS.** Tables 1 and 2 summarize the clinical observations made on each patient in the 3 groups during their febrile period. As noted, there are 3 degrees of symptoms recorded: those definitely present (+), those definitely not present (0), and those in which the degree is recorded as questionable (?). It was found that this latter category had to be included, because in so many cases the symptoms were so slight or of such brief duration that opinions as to its definite presence varied between the different observers or even by the same observer at different times.

Table 1 shows the averages for the number of paroxysms reaching 104° F. or more; the number of hours of fever experienced above 103.6° F.; the lowest hemoglobin level; and the % of weight lost in each of the 3 groups, with separate averages for the vivax and quartan infections in each group. These criteria give some indication of the severity of the malaria infection in each group.

It is noted that in those cases receiving quartan malaria therapy, the average number of hours of fever above 103.6° F. is much greater than in the vivax group, even though the average number of paroxysms experienced with each species is about the same. This, of course, is to be expected from the type of fever plateaus commonly observed in quartan infections. A significant observation, illustrated by the averages listed in Table 1, is that the

malaria in Groups B and C was a more severe disease than in Group A. The average number of paroxysms and the average number of hours of fever at the established level in Groups B and C were definitely greater than in Group A; hemoglobin values fell to lower levels than in Group A, and weight loss was greater than in Group A.

with malaria therapy were about equal in all groups.⁷ The patients in Group C seemed to remain in a somewhat better general condition, complaining less of weakness and fatigue during their paroxysms than did those in Groups A and B. This observation, however, was difficult to evaluate objectively and is, therefore, not included in the tables.

TABLE 1.—A COMPARISON OF AVERAGE VALUES FOR THE DURATION OF FEVER, LOWEST HEMOGLOBIN LEVEL, AND % WEIGHT LOSS IN GROUPS A, B, AND C

Average of:	No. paroxysms above 104° F.	No. hours fever 103.6° F.	Hemoglobin lowest level (gm.)	Weight loss (%)
Group A—Vivax	15 3	58 0	7 8	7.1
Quartan	8 8	53 8	9 0	8 5
Total	13 6	57 0	8.1	7 4
Group B—Vivax	17.7	63 4	7 9	7 7
Quartan	16 0	100 6	7 6	11 6
Total	17 2	72 7	7 8	8 7
Group C—Vivax	18 0	62.7	7.9	8 7
Quartan	15 2	95 4	7 5	10 6
Total	17 3	70 8	7 8	8 4

TABLE 2.—COMPARISON OF CLINICAL OBSERVATIONS DURING THE FEBRILE PERIOD IN GROUPS A, B, AND C

Degree:	Abdominal pain	Loss of appetite	Nausea	Vomiting	Jaundice	Liver	Spleen	Edema
	+ ? 0	+ ? 0	+ ? 0	+ ? 0	+ ? 0	+ ? 0	+ ? 0	+ ? 0
Group A—								
Vivax . .	3 0 12	12 2 0	7 4 3	2 7 5	7 1 6	8 2 5	9 2 4	3 0 11
Quartan . .	1 0 4	3 2 0	1 1 3	1 0 4	1 0 4	1 1 3	0 0 5	2 0 3
Total—No.	4 0 16	15 4 0	8 5 6	3 7 9	8 1 10	9 3 8	9 2 9	5 0 14
% .	20 0 80	79 21 0	42 26 32	16 37 47	42 5 53	45 15 40	45 10 45	26 0 74
Group B—								
Vivax . .	3 0 12	8 6 1	0 11 4	1 9 5	8 2 5	4 5 6	10 3 2	2 1 12
Quartan . .	0 0 5	2 3 0	0 1 4	0 0 5	1 1 3	3 0 2	2 0 3	1 0 4
Total—No.	3 0 17	10 9 1	0 12 8	1 9 10	9 3 8	7 5 8	12 3 5	3 1 16
% .	15 0 85	30 45 5	0 60 40	5 45 50	45 15 40	35 25 40	60 15 25	15 5 80
Group C—								
Vivax . .	3 0 12	10 5 0	4 6 5	5 4 6	9 0 6	1 3 11	11 1 3	5 0 10
Quartan . .	1 0 4	2 3 0	0 3 2	0 3 2	0 0 5	0 2 3	3 0 2	1 0 4
Total—No.	4 0 16	12 8 0	4 9 7	5 7 8	9 0 11	1 5 14	14 1 5	6 0 14
% .	20 0 80	60 40 0	20 45 35	25 35 40	45 0 55	5 25 70	70 5 25	30 0 70

+ indicates the definite presence of the symptom or palpability of the organ at some time during the febrile course. ? indicates the minimal or questionable presence of the symptom or questionable palpability of the organ at some time during the febrile course. 0 indicates the absence of the symptom or non-palpability of the organ at any time during the febrile course.

Table 2 is a summary table showing the number of patients, subdivided into vivax and quartan cases in each group, with the various degrees of symptoms. There is no striking difference in the frequency of any of the observations in the 3 groups, except for the palpability of the liver. Complications of various types, other than liver dysfunction, occurring in connection

In both Groups B and C, the distaste for "protein cocktails" was uniform. It is difficult to mix a tasty preparation of a high protein supplement including any of the specially concentrated protein compounds. However, in 1 recorded period of observation of 1 month, the patients averaged 32 gm. of protein supplement daily from the "protein cocktails" alone.

There was no very striking difference in the incidence of abdominal pain and digestive symptoms in the 3 groups. Nausea and vomiting had to be separately considered, since certain patients did not experience nausea, but would vomit each time they attempted to eat. Nausea was of lesser severity in Group B than in either of the other groups, but this slight variation may have resulted from the small size of the groups involved. In view of the fact that the malaria infections in Groups B and C were definitely more severe than in Group A, the slightly lower frequency of digestive symptoms in these groups may have greater significance.

Clinical jaundice appeared about equally in all 3 groups, and was usually slight and limited to the sclerae. Jaundice was observed in a larger number of cases than indicated by the icterus index and van den Bergh determinations. This, of course, is not surprising, since it may have been observed as a transient phenomenon during the very frequent clinical observations, and yet have been missed in the chemical determinations made at less frequent intervals. Furthermore, observations of minimal jaundice may at times be quite confusing, particularly near the end of the febrile course. At that stage, because of the generalized pallor, slight degrees of jaundice are difficult to evaluate. In addition, some of the patients still had residual skin discoloration from long periods of suppressive atabrine therapy in overseas theatres. At any rate, there was no definite correlation between the clinical observation of jaundice and the palpability of the liver.

The most interesting clinical observation concerned the palpability of the liver. The number of patients with enlarged livers in Group C was definitely less than in either Groups A or B, as noted in Table 2. In only 1 case (5%) in Group C was the liver definitely palpable, as contrasted to 7 cases (35%) in Group B and 9 cases (45%) in Group A. On the other hand, in 14 cases (70%) in Group C, the liver was definitely not palpable during

the entire course of malaria; whereas in Group B there were only 8 cases (40%) and in Group A 8 cases (40%) in which the liver was never palpable. However, there was no definite relation of digestive symptoms to the size of the liver. The difference in liver palpability was in contrast to the similar incidence of digestive symptoms in each group. It is, therefore, probable that digestive symptoms are not entirely either a cause or a result of liver enlargement.

Furthermore, the absence of liver enlargement in Group C is not due merely to the fact that this group had the highest calorie intake from the additional intravenous glucose, because the % weight loss in this group was similar to that in Group B and more than that in Group A. There is, therefore, the possibility that the addition of intravenous glucose in Group C's regimen had some relation to the observation that the liver was least often palpable in this group. If it is assumed that enlargement of the liver is *prima facie* evidence of some liver derangement, perhaps of fatty metamorphosis, one can conjecture that such involvement of the liver was least in Group C.

After the completion of each malaria course, the parasitemia was terminated with atabrine therapy consisting of 0.2 gm. of atabrine every 6 hours for 5 doses, followed by 0.1 gm. 3 times daily for 6 days—a total dose of 2.8 gm. of atabrine. After antimalarial therapy had been completed, most of the patients received 10 daily injections of mapharsen (0.06 gm. daily). In 6 cases (4 in Group A, 1 each in Groups B and C), the liver became definitely more enlarged after the mapharsen injections had been started. In 1 other case, in Group C, the liver became questionably palpable following atabrine therapy, even though this particular patient did not receive mapharsen.

In 270 cases of neurosyphilis treated with therapeutic malaria prior to the controlled study herein reported, the liver was found to be palpable in 24.7% at some time during the clinical course. This

less frequent enlargement can be explained only on the basis of infrequent and less careful examinations.

The spleen was palpably enlarged slightly more often in Groups B and C than in Group A. This may have been the result of the increased severity of malaria in these groups, as previously noted. The fact that the frequency of enlargement of the liver in the 3 groups varied in the opposite direction from the frequency of splenic enlargement supports the belief that the regimen, on which Group B and particularly Group C were placed, may have prevented liver enlargement.

various laboratory determinations in the 3 groups before, during, and after the febrile course of malaria. The average icterus index during the febrile period was slightly, though not significantly, higher in Group A than in Groups B and C. There were very few icterus index readings in the range of clinically discernible jaundice.

(b) *The van den Bergh Reaction.* The van den Bergh determinations were performed by the method of Goodrich and Gibson. Table 3, line B, shows that, again, determinations were very similar in the 3 groups.

TABLE 3.—LABORATORY DETERMINATIONS SHOWING THE RANGE AND AVERAGE OF THE 20 CASES IN EACH GROUP BEFORE, DURING, AND AFTER THE FEBRILE PERIOD

	Group A		Group B		Group C	
	Range	Average	Range	Average	Range	Average
Icterus index:						
Before	3 2-17 7	8 9	2 4-14 3	8 2	5 3-11 4	8 3
During	5 6-38 1	12 3	4 5-24 0	10 3	5 6-23 5	11 0
After	4 2-11 1	7 1	4 6-11 9	7 2	4 0-16 0	7 4
Indirect van den Bergh:						
Before	N*- 1 95	0 75	N- 1 44	0 65	N- 1 7	0 60
During	N - 3 80	1 25	N- 4 00	1 06	N- 2 95	1 16
After	N - 0 81	0 27	N- 0 90	0 28	N- 0 71	0 26
Hippuric acid excretion:						
Before	2 0- 4 8	3 6	1 0- 4 7	3 5	1 8- 5 2	3 4
During	1 2- 5 4	3 1	0 2- 5 3	3 1	0 9- 5 2	3 0
After	0 1- 5 3	2 4	0 2- 4 5	2 2	0 1- 5 2	2 5
Plasma proteins:						
Before	6 6- 8 3	7 6	6 6- 9 1	7 5	7 1- 8 9	7 6
During	5 6- 8 2	6 5	4 9- 7 8	6 4	5 5- 8 0	6 5
After	6 3- 8 5	7 2	5 9- 8 8	7 1	5 6- 8 5	7 2
Albumin:						
Before	3 9- 6 2	5 2	3 6- 5 8	4 8	4 0- 5 7	4 9
During	2 7- 4 5	3 6	2 6- 4 1	3 5	2 5- 4 5	3 6
After	3 3- 5 1	4 4	3 1- 5 5	4 3	3 3- 5 7	4 4
Globulin:						
Before	1 8- 4 3	2 7	2 0- 3 9	2 7	2 0- 4 2	2 7
During	1 6- 4 1	2 9	1 6- 4 2	3 0	1 8- 4 3	3 0
After	2 0- 4 2	2 8	1 9- 4 2	2 8	2 0- 3 7	2 8

*N signifies normal bilirubin determination, but too low to read accurately. In computing the averages, this value was taken to be 0.2 mg. per 100 cc.

Edema was observed in about the same frequency in Groups A, B, and C. In all cases, it was only minimal. In none of the edema cases were there any cardiac or urinary abnormalities to suggest either as an etiologic factor in the production of this complication.

2. LABORATORY STUDIES. (a) *Icterus Index.* Icterus index determinations were done by the method of Bernheim. Table 3 shows the ranges and averages of the

Some of the determinations for the indirect van den Bergh reaction in the period before the onset of fever were slightly high for the value considered to be normal. The same was true of several icterus index, cephalin cholesterol flocculation, and urinary urobilinogen determinations. The explanation for this may be that these laboratory studies were usually made after the inoculation of the patient with malaria parasites. Most of them, there-

fore, were studied during the pre-patent or incubation periods, during which time alterations to be observed during the febrile course may have already been initiated. At any rate, in none of the cases showing abnormal determinations during the pre-febrile period was there any clinical evidence of preëxisting liver disease. Furthermore, in all of these cases, subsequent determinations after the febrile period, and in some instances even during the febrile period, were within the normal range.

There was no apparent correlation between the average bilirubin determinations before, during, and after the febrile period and the palpability of the liver. In addition, there was no particular difference in the incidence of elevated blood bilirubin in patients with various degrees of liver

Bergh readings (above 1 mg. per 100 cc.) were uniformly encountered. From the reverse aspect, however, not all of the van den Bergh determinations above 1 mg. per 100 cc. were associated with diphasic or direct reactions. Table 5 shows that the diphasic van den Bergh reactions were most frequent in cases with liver enlargement. These observations indicate that hyperbilirubinemia was not entirely due to hemolysis, but in at least some instances to hepatocellular disease, particularly in those cases with manifest jaundice and enlargement of the liver.

A correlation of digestive symptoms with blood bilirubin levels in Table 6 shows that the incidence of definite loss of appetite increases with the bilirubin level. With values below 1 mg. per 100 cc. only 40% of patients had marked loss of appe-

TABLE 4.—INCIDENCE OF ELEVATED BILIRUBIN AND CHEMICAL MANIFEST JAUNDICE RANGE IN RELATION TO DEGREE OF LIVER ENLARGEMENT

Liver		Elevated bilirubin		Manifest jaundice	
Palpability of liver	No. cases	Bilirubin above 1 mg./100 cc. Icterus index above 12 units		Bilirubin above 2.6 mg./100 cc. Icterus index above 18 units	
		No.	%	No.	%
+	17	11	64.7	5	29.4
?	12	9	75.0	2	16.6
0	27	16	59.2	4	14.8

For explanation of +, ?, and 0, see Key, Table 2.

enlargement (Table 4). However, elevated bilirubin beyond the manifest jaundice level (2.6 mg. per 100 cc. or icterus index above 18 units) occurred twice as frequently among patients with enlarged livers as in those without increased liver palpability (Table 4). It is felt, therefore, that hyperbilirubinemia was largely due to the hemolytic effect of malaria rather than to involvement of the liver. This is, perhaps, corroborated by the fact that most of the determinations showed only an indirect van den Bergh reaction.

The direct van den Bergh reaction was diphasic, immediate, or delayed direct in only a very few instances (7 in Group A, 4 in Group B, and 5 in Group C). In these cases, in which the direct van den Bergh reaction was other than negative, the highest icterus index and direct van den

tite. In the range of latent jaundice, this increased to 72%, whereas with manifest jaundice (bilirubin levels above 2.6 mg. %), 91% of patients experienced definite anorexia. The variations of other digestive symptoms were not as pronounced.

(c) *Plasma Proteins, Albumin and Globulin Partition.* The plasma protein determinations were done by the method of Howe (Kjeldahl). Table 3, lines D, E, and F, lists the range and averages of the plasma proteins and the albumin and globulin fractions in each of the 3 groups during the 3 periods of observation. No particular difference between the groups was apparent.

The total protein decreases during the febrile period, but returns almost to normal levels thereafter. Simultaneously with this decrease, there is a slightly

greater fall in the albumin fraction and a coincident slight rise in the globulin fraction. These changes in plasma proteins during the course of malaria have been previously described by Kopp and Solomon.⁵

(d) *Hippuric Acid Excretion Test.* The hippuric acid excretion test was performed by Quick's ether extraction and digestion method. No urine specimens were sent to the laboratory for determination if the test seemed incomplete in any way.

TABLE 5.—INCIDENCE OF DIPHASIC AND DIRECT VAN DEN BERGH REACTIONS DURING THE FEBRILE PERIOD RELATED TO VARIOUS DEGREES OF LIVER ENLARGEMENT

Liver		van den Bergh reactions					
Palpability of liver	No. cases	Diphasic		Direct immediate		Direct delayed	
		No.	%	No.	%	No.	%
+	17	5	29.4	1	5.9	1	5.9
?	13	1	7.7	1	7.7	1	7.7
0	30	3	10.0	1	3.3	2	6.7

For explanation of +, ?, and 0, see Key, Table 2.

TABLE 6.—INCIDENCE OF BLOOD BILIRUBIN LEVELS AND VAN DEN BERGH REACTIONS RELATED TO DIGESTIVE SYMPTOMS

Loss of appetite	Blood bilirubin (mg./100 cc.)						van den Bergh reaction			
	Above 2.6 mg.		1-2.6 mg.		Below 1 mg.		Diphasic immediate or delayed direct		Indirect only	
	No.	%	No.	%	No.	%	No.	%	No.	%
+	10	90.9	18	72	8	40	11	68.7	25	64.1
?	1	9.1	7	28	11	55	5	31.3	13	33.2
0	0	0	0	0	1	5	0	0	1	2.5
Nausea										
+	4	36.3	4	16	3	15	5	31.3	6	15.3
?	5	45.4	12	48	7	35	6	37.4	18	46.1
0	2	18.1	9	36	10	50	5	31.3	15	38.4
Vomiting										
+	2	18.1	3	12	3	15	3	18.7	5	12.8
?	5	45.4	12	48	4	20	7	43.7	14	35.9
0	4	36.3	10	40	13	65	6	37.5	20	51.2

For explanation of +, ?, and 0, see Key, Table 2.

It is interesting that in malaria, the drop in serum albumin becomes apparent so rapidly, while in diseases with obvious loss of albumin or decreased protein intake, the reflection in the serum level seems to appear much slower. It seems unlikely that decreased protein intake and depleted protein stores are the entire explanation in the malaria, but perhaps the presence of liver disease in some way inhibits the formation of serum albumin from its precursors. The rapid return of protein values to normal levels after the febrile period also supports this hypothesis.

Nurses were instructed to stand at the patient's bedside until the sodium benzoate was ingested. If it was incompletely taken, if there was any vomiting after its administration, or if there was any question that the total 4 hour specimen of urine was not collected, then such tests were discarded.

Table 3, line C, shows the range and averages of the hippuric acid determinations in the various periods in the 3 groups. The results were similar in all groups. However, although during the febrile period the hipuric acid excretion drops (a

fact not unexpected), the average excretion continues to drop during the period after the termination of fever. This is hardly to be expected in view of the return towards normal of all the other laboratory findings, and requires further comment.

We must assume that the results are factual, even though a number of determinations after the febrile period, and a few during fever, were very low (0.1 to 0.8 gm.), because the techniques were checked both on the ward and in the laboratory.

function of the liver, the use of these drugs may account for the increasing impairment of the detoxification function, as reflected by the hippuric acid excretion test. A comparison of the averages of the hippuric acid determinations with the degree of liver enlargement reveals no correlation of these findings.

(c) *Cephalin Cholesterol Flocculation Test.* This determination was done by the method of Hanger, using Wilson Laboratory Antigen. Table 7 shows the readings of the cephalin cholesterol flocculation test

TABLE 7.—CEPHALIN CHOLESTEROL FLOCCULATION REACTIONS (48 HOUR) ON THE 20 PATIENTS IN EACH GROUP BEFORE, DURING, AND AFTER THE FEBRILE PERIOD

		No. cephalin cholesterol flocculation reactions					
		Neg.	+	+	++	+++	++++
Group A—Before		4	2	5	3	1	1
	During	2	0	0	0	2	30
	After	10	0	1	2	6	15
Group B—Before		7	1	2	4	1	2
	During	2	0	0	0	0	31
	After	6	2	3	0	9	15
Group C—Before		6	1	3	5	1	1
	During	1	0	0	0	2	32
	After	7	3	0	3	4	17

TABLE 8.—URINARY UROBILINOGEN DETERMINATIONS ON THE 20 PATIENTS IN EACH GROUP BEFORE, DURING, AND AFTER THE FEBRILE PERIOD

	Group A		Group B		Group C	
	Range	Average	Range	Average	Range	Average
Before	10-40*	28	10-60	26	20-150	35
During	10-300	80	10-800	86	10-250	87
After	10-150	37	20-100	41	20-100	35

* These figures indicate the dilution in which the urobilinogen test was positive, *e. g.*, 40 indicates urobilinogen was positive in 1:40 dilution.

There is a possible explanation, however, as to why the detoxification function may be increasingly impaired after the febrile period. As previously noted, in at least 7 cases the liver enlargement was found to increase after the termination of malaria with atabrine and after the 10 daily injections of mapharsen. Furthermore, the hippuric acid excretion did not drop to the extremely low levels noted above in any of the cases not receiving mapharsen after the febrile course. All of the extremely low results occurred in patients receiving the 10 daily injections. Because both atabrine and mapharsen place an added load on the detoxifying

in 48 hours in the various periods in each group. The readings were entirely similar in all 3 groups. The reaction was 4+ in 48 hours in practically every case of malaria during the febrile period. In only 2 of the 60 cases included in this study did the reaction not reach 4+ in 48 hours. These 2 patients were both in Group A; both had quartan malaria; and both showed only a 3+ reaction in the one reading made in each case during the febrile period. One patient experienced only 8 hours of fever above 102° F., and then developed a spontaneous remission. He was subsequently reinoculated with falciparum malaria in order to complete

the desired therapeutic course. The other experienced only 4 hours of fever above 103.6° F. He had no paroxysms reaching 104° F. but did run a low grade fever for 32 days.

The cephalin cholesterol flocculation tests did not return to normal levels as rapidly as the other laboratory findings. As a matter of fact, at the time of discharge from the hospital, which in most cases was between 3 and 4 weeks after the termination of the malaria, about 50% of the patients still had a flocculation reading of 4+ in 48 hours. This may be an indication that the liver had not returned to its normal state, and such patients should be further followed.

(f) *Urinary Urobilinogen Determination.* This test was performed by Wallace and Diamond's modification of Ehrlich's method. Table 8 shows very little difference between the range and averages of the determinations in the 3 groups during the different periods of observation.

(g) *Prothrombin Time Determination.* Prothrombin determinations were done by the method of Quick, using thromboplastin made by the Fourth Service Command Laboratory and by the Finney General Hospital Laboratory. The prothrombin time determinations were reported in seconds. Though each group of prothrombin readings were performed with lots of thromboplastin which gave normal readings with normal blood when prepared, there was enough variation between the various lots of thromboplastin to make the determinations more dependent upon the particular lot used and the day the test was done than upon any condition in the patient. For this reason, the only conclusion drawn from these results was that they cannot be reported in seconds if it is desired to compare the determinations made at different times with different lots of thromboplastin on the same or different patients. The results must be reported by some method which allows comparison between various lots of thromboplastin, such as the percentage method outlined by Hurn, Barker and Magath.²

3. HISTOLOGIC OBSERVATIONS. During the course of this study, 2 patients not included in the investigation of liver function died during therapeutic malaria. The pathologic findings in the liver in these cases were as follows:

Autopsy Findings. CASE 336. *Death from spontaneous rupture of the spleen.*

Liver. Weight 2100 gm. The surface was dark, reddish tan and finely granular. The edges were rounded. On section the cut surface was dark, reddish tan in color and was more friable than normal. The normal hepatic pattern was present on microscopic examination, but there was moderate thickening of the peripheral connective tissue with infiltration by many round cells and occasional histocytes. The Kupffer cells were swollen and contained reddish brown granular pigment. The hepatic cells showed cloudy swelling and finely granular yellowish green pigment in their cytoplasm. Occasional foci of interstitial hemorrhage were present. The small bile ducts appeared normal. The portal veins contained several mononuclear cells.

CASE 445. *Death, primary cause unknown, related to hypocalcemic tetany.*

Liver. Weight 1575 gm. The liver appeared enlarged with some rounding of the lower edge. Color normal but consistency softer than normal. The cut edge everted slightly and the cut surface was homogeneous, reddish brown in color and moderately congested. The lobules were fairly well demarcated. Microscopic examination showed a capsule of normal thickness but generalized enlargement of the hepatic lobules resulting from dilatation of the sinusoids with blood. The sinusoids contained an increased number of mononuclear cells. The histiocytic endothelial cells showed moderate proliferation. The mononuclear cells assumed ameboid shapes, contained a few vacuoles and some granular yellow pigment. The hepatic cells in the liver cord appeared compressed. The central veins displayed congestion and slight subintimal infiltration by mononuclear cells and lymphocytes. The periportal connective tissue showed an increased number of fibroblasts in some areas, resembling a submiliary granuloma. The bile ducts contained a moderate amount of bile.

The increased connective tissues in the periportal spaces and pigment deposition were common findings in the 2 livers. In 1 case, the hepatic cells showed cloudy swelling and occasional foci of interstitial hemorrhage, while in the other, the hepatic cells were compressed, apparently from dilatation of the sinusoids with blood.

These anatomic findings are to be compared to those reported by Kern and Norris.³ In their 2 cases of naturally acquired malaria, who died of this disease, the findings in the liver at postmortem examination revealed (1) hepatitis, with central and mid-zonal necrosis, and (2) central and mid-zonal atrophy. In 4 cases of acquired malaria, who died of other causes, they found swelling of the parenchymal liver cells and consequent compression of the sinusoids.

From these histologic observations, it can be stated that there is definite evidence of some alteration of structure in the liver during malaria infections. Observations are insufficient to categorize the type of involvement.

Summary. From the results of this study, it appears that the liver is involved in every case of malaria, showing some degree of dysfunction by various tests. The function of detoxification, as measured by the hippuric acid excretion test, was depressed for the longest period,

although atabrine and mapharsen administered after malaria therapy may contribute to the continued depression of this function. The involvement of the liver is probably not very severe, since other liver function tests return to normal in a short period after the termination of malaria. The cephalin cholesterol flocculation test, however, returns to normal more slowly than the other tests performed in this study.

This study also indicates that the enlargement of the liver is no indication of the degree of dysfunction. Furthermore, the digestive symptoms experienced during malaria therapy are not dependent upon the liver disease, but apparently largely upon the malaria, probably the fever, itself. Though marked loss of appetite increased in frequency as the blood bilirubin became elevated, the frequency of definite anorexia did not vary significantly with liver enlargement.

Judging by the liver function tests used in this study, there was no striking protection of the liver by the addition of protein (amounting to more than 32 gm. daily) to the diet, high vitamin intake, or crude liver extract injections. However, the addition of 75 gm. of glucose intravenously daily did decrease the incidence of liver enlargement quite significantly.

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EFFECT OF ROOM TEMPERATURE ON SEDIMENTATION RATE OF RED BLOOD CELLS OF MAN*

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THIS paper is an account of experiments made for the purpose of discovering how the sedimentation rate of red blood cells is changed by room temperature, and if it is possible to make a graph which will give the true sedimentation rate at a given base temperature, for example 20° C. in 1 hour in a young man would be only 8 mm. at 20° C., which is a normal rate for the test of Wintrobe and Landsberg, where the range for healthy men is 0 to 10. In the same way use of a sedimentation rate in which a correction has not been made for the effect of tempera-

TABLE 1.—SEDIMENTATION RATES AT 6 DIFFERENT TEMPERATURES

Unrighted sed. rate, mm.:hr.:	55-50	49-45	44-40	39-35	34-30	29-25	24-20	19-15	14-10	9-0
22° C.—N.O.	11	13	14	7	14	21	12	18	17	29
M.	47.0	37.6	31.0	25.3	17.6	14.6	10.7	7.0	4.0	2.4
C.V.	10.0	18.6	14.5	15.0	25.0	24.8	15.9	28.6	35.0	45.8
26° C.—N.O.	9	4	8	5	2	5	7	9	9	16
M.	48.2	38.2	31.2	26.0	18.0	15.0	12.0	7.3	5.0	3.0
C.V.	12.0	13.4	10.9	15.4	23.3	26.7	14.2	23.4	32.0	33.3
30° C.—N.O.	9	13	13	7	14	21	12	18	17	29
M.	49.7	42.0	35.0	29.0	21.6	19.0	15.0	10.5	6.5	3.9
C.V.	8.0	9.5	8.3	9.7	20.8	14.7	9.3	18.1	26.2	41.0
34° C.—N.O.	19	5	8	5	2	6	8	10	9	17
M.	52.0	43.6	37.0	32.4	25.4	21.0	17.5	12.8	8.2	4.9
C.V.	7.7	6.9	6.8	6.2	23.2	17.1	8.0	13.3	15.7	28.6
37° C.—N.O.	11	9	12	6	10	15	10	14	14	23
M.	52.0	43.2	38.0	33.5	27.4	22.0	18.2	13.4	9.0	5.3
C.V.	6.4	8.8	5.3	6.6	9.5	9.1	7.7	11.2	13.3	26.4
41° C.—N.O.	9	9	9	5	5	13	9	13	12	19
M.	53.8	47.0	42.6	37.4	31.5	27.0	22.4	17.0	12.0	6.0
C.V.	6.2	2.1	3.2	2.2	5.4	5.2	4.5	8.8	8.8	28.4

Number of observations (N.O.), mean sedimentation rates (M.), and coefficients of variation (C.V.).

Though we have had knowledge for a long time that room temperature may have an effect on the sedimentation rate of blood, sufficient attention has not generally been given to this possible cause of error. DeCourcy,¹ Fahreus,² Gordon and Cohn,³ Rosenthal,⁴ Stats and Wasserman,⁵ and Westergren⁶ have gone into certain details of this question, but have not made graphs for correction of the error. Graph 2 in this paper makes clear the effect of high room temperature on sedimentation rate. For example, at 37° C., a sedimentation rate of 16 mm.

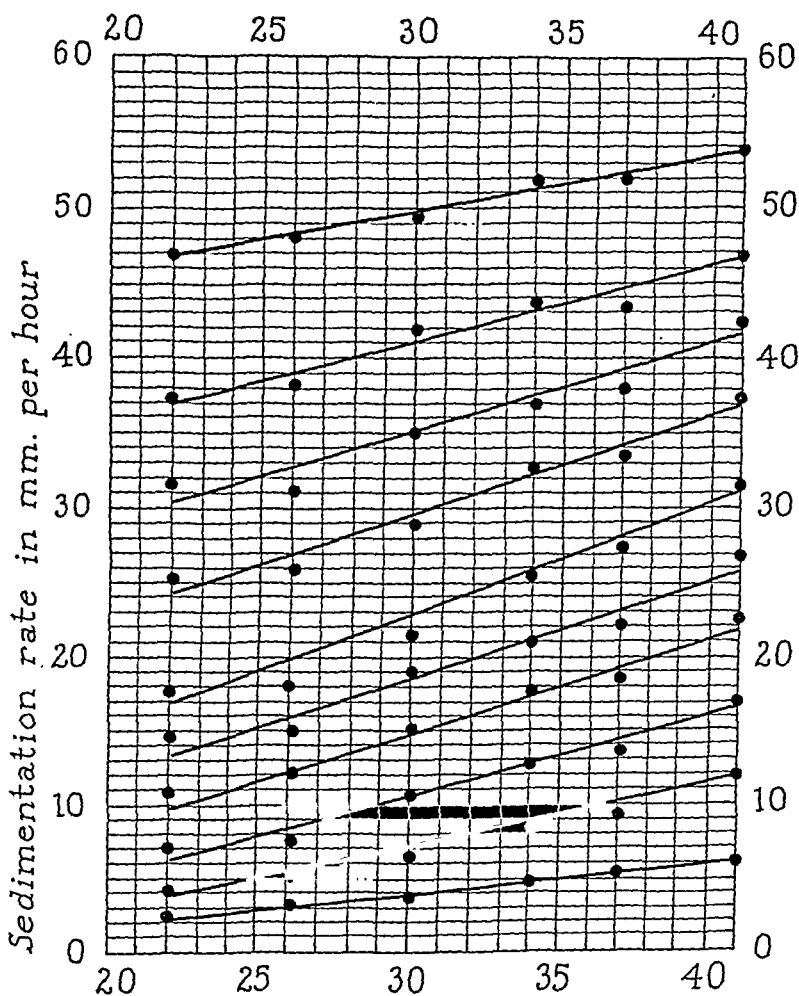
ture may be the cause of an error in diagnosis of patients, who have been under medical care for a long time, and at different times of year. Comparison of a sedimentation rate measured on a warm day in summer with one measured on a cold day in winter may be the cause of a wrong opinion of the patient's condition. But, if a correction is made to 20° C., which is normal "room temperature," then comparison of observations made at different temperatures is possible.

* This paper is in Basic English. But for a small number of special scientific words, only words in the basic and international science lists are used. The special words are: diagnosis, hematocrit, sedimentation, standard deviation.
† On leave of absence from the Institute of Pathology, Western Reserve University, and University Hospitals of Cleveland.

Materials and Ways of doing the Experiments. As much venous blood was taken from 160 patients as was necessary for measuring the red blood cell sedimentation rate by Wintrobe and Landsberg's method⁷ at 6 different temperatures. Selection was made of this method because it is widely used and because it gives other observations

0.5°C. ; $30^{\circ}\pm 0.5^{\circ}\text{C.}$; $34^{\circ}\pm 0.5^{\circ}\text{C.}$; 37°C. (range between 36° and 37°C.); and $41^{\circ}\pm 0.5^{\circ}\text{C.}$

Blood was put in glass vessels at body temperature, and observations were made at the temperatures given above. Correction was made for hematocrit errors at room



GRAPH 1.—Temperature in degrees centigrade.

of value, for example, hematocrit. The patients were all young, white men 18 to 30 years old, who were sick with a number of diseases. No record was made of the diseases, as the cause of the high sedimentation rate was not important for our purposes.

Observations were made at these temperatures: 22°C. , or room temperature (this was in fact between 20° and 23°C.); $26^{\circ}\pm$

temperature at the end of every test in the normal way.

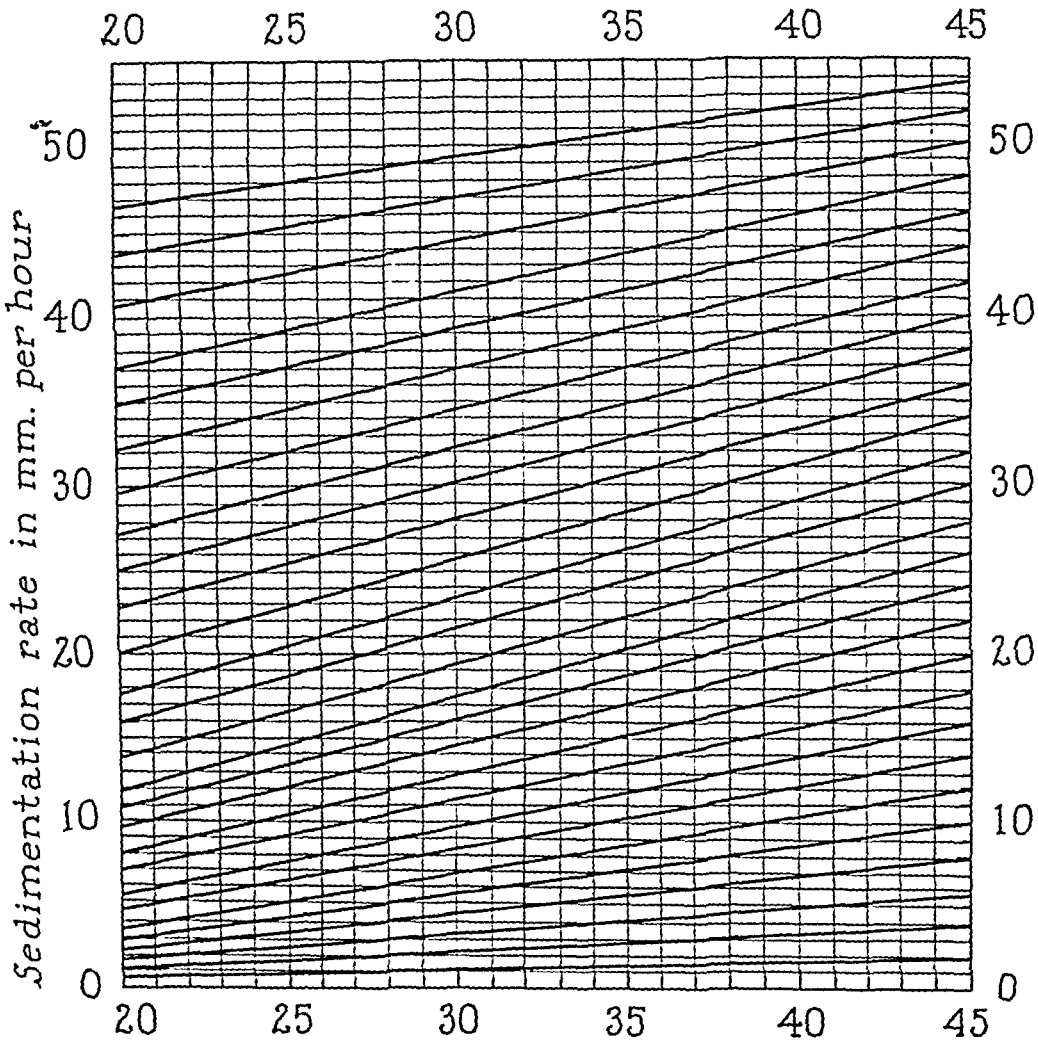
Observations. A small number of tests made it clear that temperatures between 15° and 22°C. do not have an important effect on the sedimentation rate, and for this reason no other observations were made at temperatures lower than 22°C.

One hundred and sixty sedimentation rates.

were measured. At 22° C. the values were in the range 0 to 60 mm. in 1 hour, whereas at higher temperatures the values were increased. In general, it may be said that the higher the room temperature the quicker the sedimentation rate, and that this relationship may be put in the form of a straight line. To make a statistical analysis of the observations, all readings at 41° C. were put in groups of 5 mm., and means for each

graph paper and making a straight line through the points. Other lines were then put in place on the base line at distances of 2 mm. (Graph 2).

The process of making Graph 2 must be written about at greater length. Means of the experimentally observed sedimentation rates are recorded in Graph 1, from which we see that it is possible to make a straight line through the points in such a way that



GRAPH 2.—Temperature in degrees centigrade.

group were worked out with standard deviations and coefficients of variation. Like values were used for each of the other 5 temperatures (Table 1). Readings at 41° C. were taken as a starting place or base line for making graphs, because we had the desire to see what the true sedimentation rate of blood would be at 20° C. Graphs were made by putting the means of the sedimentation rates for each temperature on

there is good distribution of points over and under the line. In fact, the distance of these points from the lines is no greater than the coefficient of variation of the means. But it is seen that all lines are not parallel. Probably the reason for this is that, as the sedimentation rate is increased, red blood cells are pushed tighter and tighter together, and the lines get more and more nearly parallel with the base. In other

words, in a number of conditions complete or nearly complete sedimentation of cells may take place so quickly that temperature will have but a small effect. Though these facts are so, parallel lines may be placed between those made from the experimental observations without causing a greater error than is present in the test itself. In this way a graph can be made for correction of errors caused by changes in room temperature.

From Table 1 it is seen that at low temperatures sedimentation rates in the normal range have a high coefficient of variation. This is because the measurements are in millimeters so that a difference of 1 mm. which makes little change, for example, in a mean of 30 mm. will make a great change in a mean of 4 mm. or less.

Use of Graph. To make use of the temperature correction graph, the sedimentation rate is measured in the normal way. If the room temperature is high the value at 20° C. is given by looking on the graph for the point where room temperature and sedimentation rate come together. Now go down the nearest sloping line as far as 20° C. where the true sedimentation rate is seen. For example, if the sedimentation rate is 22 mm. in 1 hour

at 38° C., the sedimentation rate at 20° C. will be 12 mm. After making the necessary temperature correction, the hematocrit error may be overcome by using the graph made by Wintrobe and Landsberg.⁷

Chief Points of Paper. 1. Observation was made of the effect of high room temperature on sedimentation rate of red blood cells as measured by Wintrobe and Landsberg's method.

2. A high room temperature, such as may be present in warm weather or in an overheated room, may be the cause of an important increase in sedimentation rate, which may make for an error in diagnosis.

3. A graph for correcting the effect of temperature was made from measurements of sedimentation rate at 6 different temperatures (22°, 26°, 30°, 34°, 37° and 41° C.) in 160 sick men 18 to 30 years old.

4. Use of the graph will give the true values at a given base temperature of sedimentation rates which have been measured at a higher temperature (20° to 45° C.). The suggestion is made that 20° C. be used for this base temperature.

5. If corrections are made for the errors which are caused by temperature and hematocrit, then comparison can be made of sedimentation rates measured at different room temperatures.

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APLASTIC ANEMIA IN SOLDIERS TREATED WITH ATABRINE (QUINACRINE)

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DRUG suppressive treatment of malaria insured the success of our military operations in the South and Southwest Pacific Areas where many of the islands are among the most highly malarious regions in the world. It also made possible maintenance of our armed forces in certain areas of the Asiatic (China-Burma-India) Theater. Bitter experiences early in the war proved that malaria could neutralize our combat forces far more rapidly and effectively than enemy fire. Other means of malaria control, apart from the use of protective clothing and mosquito repellents, were not feasible until the units were firmly established.

Although drugs naturally did not prevent infection, the dose of atabrine routinely employed for suppressive treatment (0.6 to 0.7 gm. per week in daily doses of 0.1 gm. each) seemed to cure falciparum malaria unless continued reinfection occurred. The symptoms of vivax malaria were suppressed in most cases as long as therapy was continued. Consequently suppressive treatment was carried on until infected individuals were in a situation where active treatment was possible.

Atabrine proved superior to quinine for suppressive treatment, being more effective and better tolerated by the troops. It was generally regarded as harmless and, for the most part, with good reason. Intestinal disturbances occasionally followed the first few doses of atabrine but usually disappeared, even though treatment was not interrupted. Sometimes quinine had to be substituted when intolerance to atabrine became evident.

The possibility that atabrine in suppressive dosage might produce harmful

effects was entertained by some observers. In an effort to determine whether this was valid, the Army Institute of Pathology instructed all laboratory officers to include any existing records of atabrine therapy in autopsy protocols no matter what the cause of death. A microscopic examination of tissues and a review of postmortem findings were carried out by staff members of the Institute. For some time nothing of significance was observed, but later it became apparent that aplastic anemia was the cause of death in a disproportionately large number of cases represented by autopsy material sent from the South and Southwest Pacific Areas, where a rigid antimalarial régime was in force. This trend was continuous. In addition, material from sternal bone marrow biopsies of a few non-fatal cases was received from the same theaters.

This report is based on a study of 57 cases of aplastic anemia with symptoms first becoming apparent when the patients were in the South or Southwest Pacific Areas, or the Asiatic (China-Burma-India) Theater. The incidence of the condition in these regions is contrasted with that in the Army elsewhere. For the sake of brevity the troops stationed in the continental and territorial United States, and all foreign theaters exclusive of the areas specified above will be designated as *Group I*, and those in the special areas as *Group II*.

INCIDENCE. The polygons in the upper section of Chart 1 represent the actual number of cases of aplastic anemia which occurred in Group I according to the month during which the initial symptoms were noted by the patients. The corresponding curve in the chart shows the

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total troop strength of Group I and is drawn to scale for comparison with the troop strength curve of Group II below. The polygons in the lower section tally the cases of aplastic anemia from Group II by month of onset.

It is apparent that aplastic anemia in Group I does not increase at a rate out of proportion to rising Army personnel, whereas the incidence in Group II mounts enormously. This disparity becomes all

apparent decline of incidence in Group II during the first half of 1945 is of dubious significance, due to incomplete figures. The corresponding value for Group I is apt to be more nearly complete, however, as virtually all laboratories in the European Theater have been closed and their cases already collected at the Institute; furthermore, material from hospitals in the United States is generally sent in

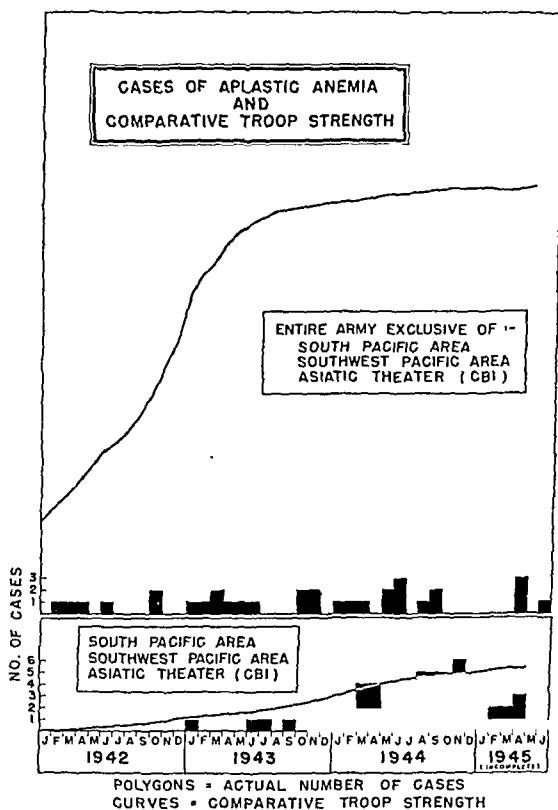


CHART 1.

the more striking when the incidence is computed semi-annually as cases per 100,000 troops (Chart 2). Group I has a rate ranging from 0.04 to 0.18 cases per 100,000, the larger figure probably being fortuitously high, as it occurred when the Army was relatively small. It has not been reached again during any subsequent $\frac{1}{2}$ year period. After an initial lag, Group II displayed a precipitous rise from 0.66 to 2.84 cases per 100,000. The

rather promptly. No data are available for the last half of 1945.

ETIOLOGY. The years 1943 and 1944 have been selected for a comparative study of the causes of aplastic anemia in both groups, since this was the period in which returns are complete and in which Group II is significantly represented. The results are shown in Chart 3.

In *Group I* aplastic anemia twice followed arsenical treatment of syphilis;

3 times, irradiation of malignant tumors; 4 times, administration of sulfonamides; in 11 cases there was no demonstrable cause. One patient from the North African Theater not included in the tabulation was said to have taken atabrine

All had received suppressive doses of atabrine over periods varying from 1 to 34 months, the majority between 4 and 14 months. The 34 month case was unique, however, and the patient apparently had not adhered to the prescribed

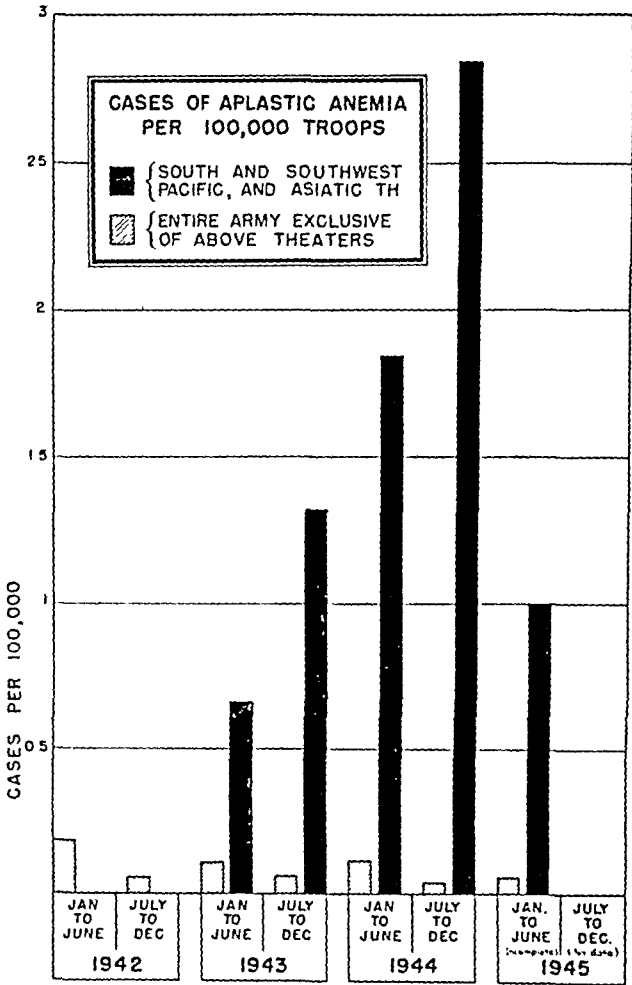


CHART 2.

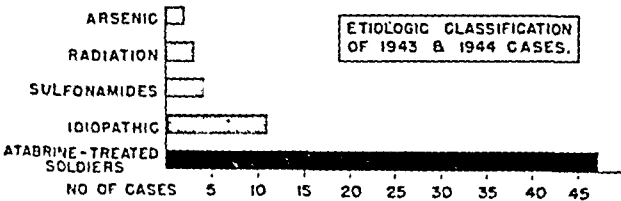


CHART 3.

during the previous 18 months, but there was no information regarding the amount or regularity of the dose. The 47 cases occurring in *Group II* during 1943 and 1944 are indicated in Chart 3 as "atabrine-treated soldiers."

régime, as his record showed repeated "break-through" of tertian malaria which required active treatment. There was specific mention of overdosage prior to the onset of illness in 6 cases. Four men had increased the dose to 0.2 gm. daily,

1 for 3 weeks, another for 6 months, a third for 8 months, and the last for an unspecified time. Another soldier took between 20 and 30 tablets during the 4 days preceding onset of symptoms; still

or detached service were given supplies of atabrine adequate for estimated time away from their units and, although instructions were given, administration was unsupervised.



FIG. 1.—Typical sternal bone marrow of a patient dying from aplastic anemia of 2½ months' duration. Marrow spaces are almost completely filled with fat, the cells sprinkled through the interstices being residual erythrocyte and granulocyte progenitors admixed with lymphocytes, plasmocytes and histiocytes. (Reduced from $\times 125$.)

another was said to have ingested "massive doses" for 3 weeks before. Other instances of overdosage probably occurred, but were either not known or not recorded. For example, men on patrol

Seven of the group treated with atabrine had also taken sulfathiazole or sulfadiazine. In 2 instances it was not possible to exclude this as a precipitating factor, but in the others it was obvious

that the drugs were administered only after the onset of the anemia and in 2 cases aggravated the purpuric manifestations. Atabrine dermatitis complex, the term recently proposed to designate so-called New Guinea lichen planus, preceded aplastic anemia in 20 cases; in these

topical applications of various sorts were used and light Roentgen ray treatment was given in 1, but these were not regarded as significant causal factors. A few patients had taken aspirin for headache which was frequently a feature of the prodromal period. The remainder had



FIG. 2.—The sternal marrow from an acute case (death in 10 days) is largely depleted and shows early fat replacement. The fat cells are small in contrast with those in Figure 3 and some are rather ragged. The remaining cells are mostly erythroblasts, normoblasts, and histiocytes. There is also hemorrhage in the interstices which in some cases is extensive. (Reduced from $\times 550$.)

FIG. 3.—An orderly and nearly complete fat replacement is noted in the marrow of a patient who survived 5 months. The small clusters of nucleated elements are chiefly normoblasts. (Reduced from $\times 550$.)

FIG. 4.—A sternal biopsy performed on this patient early in the course of the disease disclosed a fatty marrow. At autopsy 10 months later marrow spaces of all bones examined showed this extensive secondary myelofibrosis. Hemosiderin-laden histiocytes are conspicuous in the fibrillar mat. (Reduced from $\times 550$.)

received no drugs, nor could a history of exposure to other etiologic agents be elicited.

It would appear that atabrine is responsible for the *relatively* high incidence of aplastic anemia in troops given extended suppressive therapy.

The following descriptions are based entirely on material from Group II in

which atabrine is thought to have been the significant etiologic agent:

PATHOLOGY. The *bone marrow* in all cases was badly depleted of normal hematopoietic elements, often almost totally so (Fig. 1), and there was no evidence of extramedullary hematopoiesis. Usually the residual cells of the marrow belonged to the erythropoietic series and were in

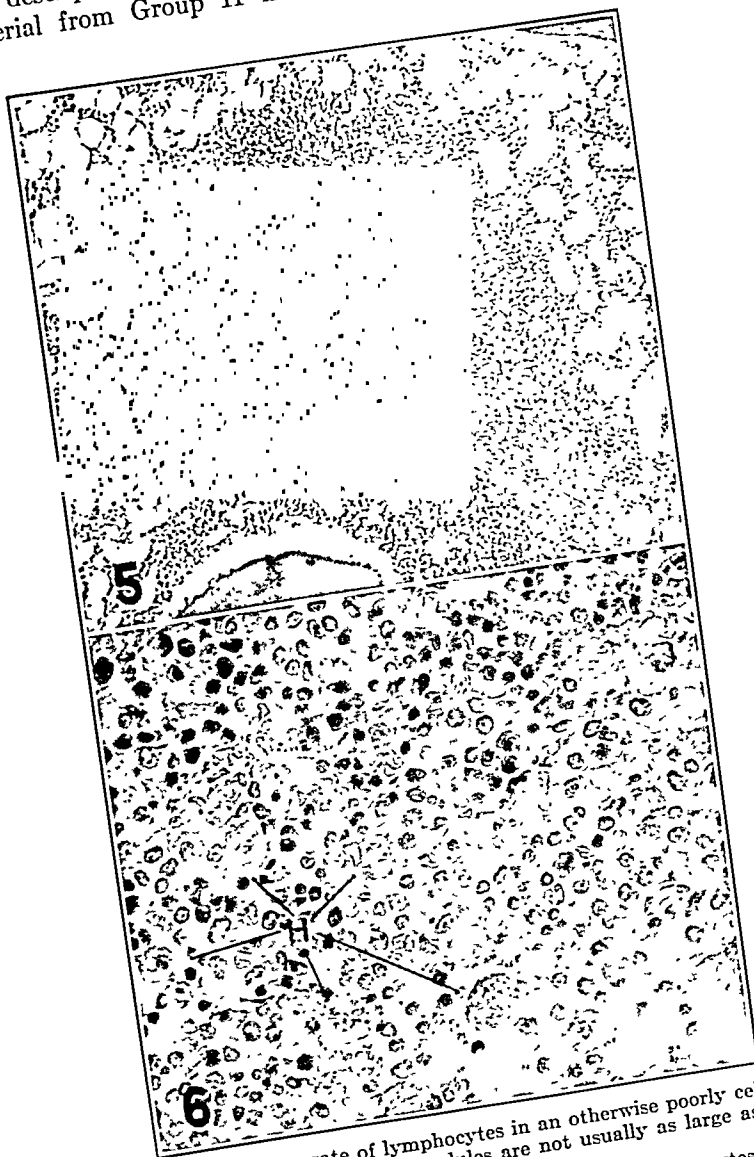


FIG. 5.—A large folliculoid aggregate of lymphocytes in an otherwise poorly cellular marrow. This is a frequent finding, although the lymphoid nodules are not usually as large as the one illustrated. (Reduced from $\times 125$.)

FIG. 6.—A more diffuse influx of lymphocytes, plasmocytes, and histiocytes (H), the histiocytes displaying marked erythrophagocytosis. This sort of infiltrate may sometimes occupy fairly large areas of the sections, but other portions disclose the basic aplastic state of the marrow. (Reduced from $\times 800$.)

the late erythroblastic and normoblastic stages. The few granulocytes were generally stab or segmented forms, although occasionally small foci of myelocytes or even younger elements were encountered. Megakaryocytes were either absent or very sparsely distributed and when present often showed evidence of degeneration. The marrow spaces were replaced by fat,

raggedly in the acute cases, neatly in those of longer standing (Figs. 2 and 3). One man who lived for 10 months and received 65 blood transfusions had extensive secondary fibrosis of the marrow cavity of all bones examined (Fig. 4). There was an influx of lymphocytes and plasmacytes (Figs. 5 to 8), slight in some cases and conspicuous in others, the lym-

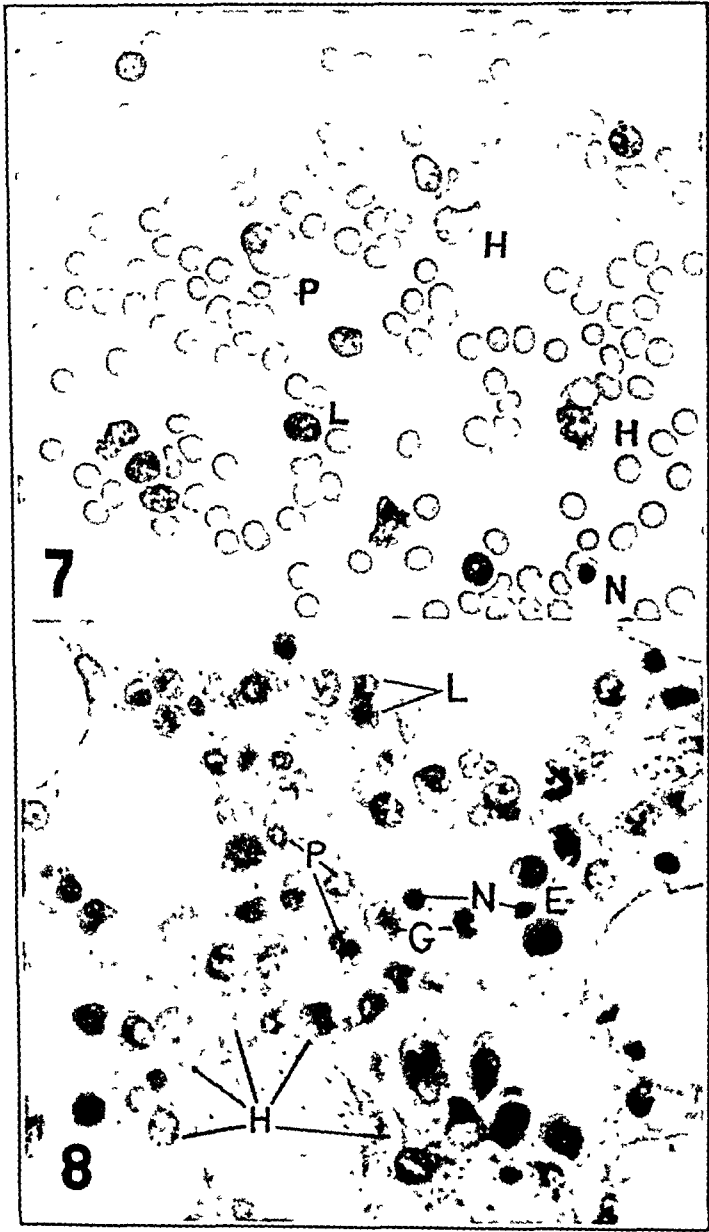


FIG. 7.—A typical sternal aspirate from a patient ill 6 months showing almost complete depletion of the marrow. Most of the cells are lymphocytes (*L*), with an interspersed histiocytes (*H*) and a plasmacyte (*P*). Hematopoietic elements are represented by the single normoblast (*N*). The histiocytes each contain a faded erythrocyte. (Reduced from $\times 600$.)

FIG. 8.—Detail of one of the residual cell clusters in the marrow of a patient who survived for 2 months. Sample cells are indicated as: *L*, lymphocytes; *P*, plasmacytes; *H*, histiocytes; *G*, granulocytes (a myelocyte and a metamyelocyte); *E*, erythroblasts (above and below the letter); *N*, normoblasts. (Reduced from $\times 800$.)

phocytes frequently aggregating to form pseudofollicles (Fig. 5). Histiocytic proliferation was usually very pronounced in patients who survived for several months and received many transfusions; most of the cells contained hemosiderin and erythrocyte fragments. Sometimes this combination of lymphocytes, plasmocytes, and

histiocytes attained such proportions that at first glance the fundamental hypoplastic state was not apparent (Fig. 6). For example, in 1 such instance the appearance of the bone marrow was interpreted as evidence of aleukemic lymphatic leukemia by the laboratory officer who submitted the case.

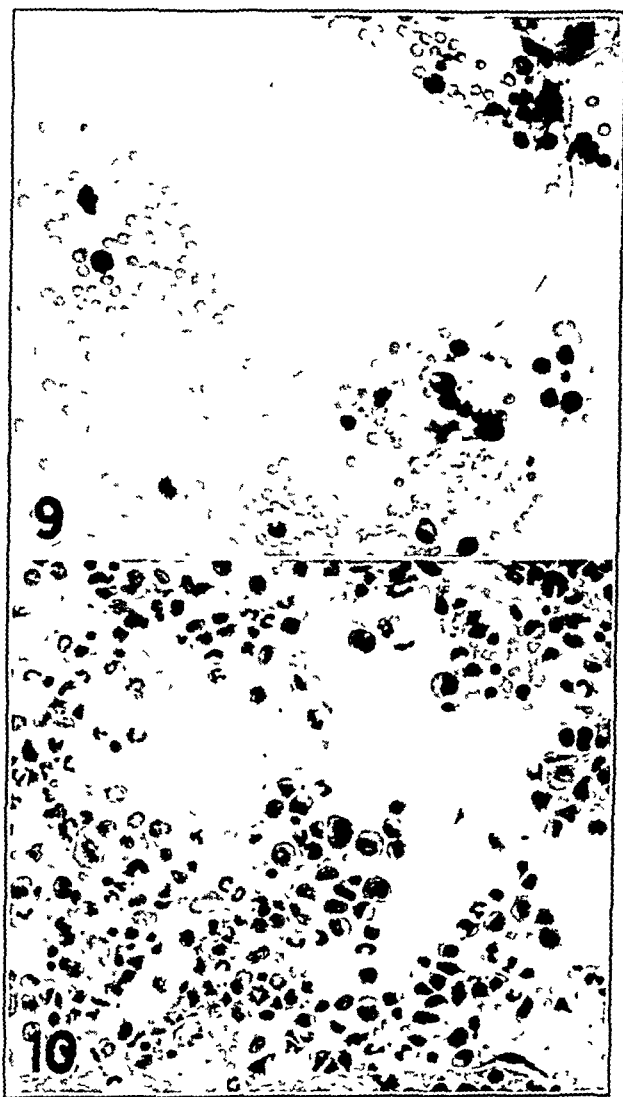


FIG. 9 Sternal aspiration disclosing a markedly hypoplastic marrow on the 17th day of illness. The 2 degenerated megakaryocytes shown in the upper right were the only ones observed in the smears. The aspirate contained considerable quantities of fat and serous fluid which blurred the preparations to some degree. (Reduced from $\times 300$.)

FIG. 10.—Aspirated marrow from the same case on the 72nd day showing a strong regenerative activity, particularly of the granulocyte series. The clinical state of the patient was correspondingly better and there was promise of complete recovery. (Reduced from $\times 300$.)

Bone marrow findings during life are described in later paragraphs dealing with sternal biopsies.

Apart from the marrow changes, *hemorrhage*, involving the skin, mucous and serous membranes, and viscera to varying degrees, was the conspicuous feature in virtually the entire series. Cerebral hemorrhage was the immediate cause of

the urinary tract was an almost invariable finding (Fig. 14).

Infection was not so prominent as one would expect in the presence of constant and profound neutropenia, probably because penicillin had been used freely in most cases. There was evidence of bacteremia in only 6, bronchopneumonia in 8, and urinary tract infection in 1.

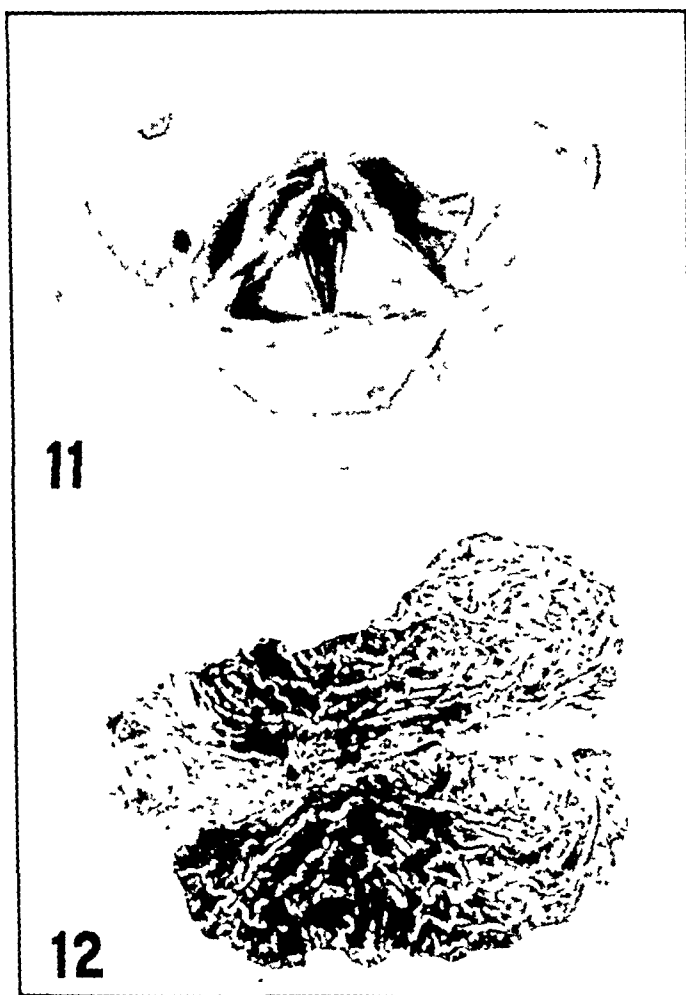


FIG. 11.—The larynx of a patient who died of asphyxia, the airway having been completely occluded. Apposition of the true vocal cords resulted from extensive submucosal hemorrhage.

FIG. 12.—Punctate and diffuse bleeding in the stomach of a patient who died soon after a series of gastro-intestinal hemorrhages. The colon presented a similar appearance with the addition of superficial sloughs

death in 10 cases (Fig. 13), occlusive laryngeal hemorrhage in 1 (Fig. 11), and massive gastro-intestinal hemorrhage in 2 others (Fig. 12). Submucosal hemorrhage in the colon was occasionally followed by surface sloughs simulating those in acute ulcerative colitis. Bleeding along

Liver lesions were observed in 10 cases (Chart 4), in 5 being indistinguishable from epidemic hepatitis as described by Lucké.¹ Active periportal hepatitis was seen in 3 cases, focal intralobular hepatitis in 1, and central phlebitis in another. The liver lesions were probably incidental,

particularly those resembling epidemic hepatitis which was widespread in the areas from which the cases were sent.

The *atabrine dermatitis complex* which preceded the anemia in 25 cases (Chart 4) is described in detail in a separate report

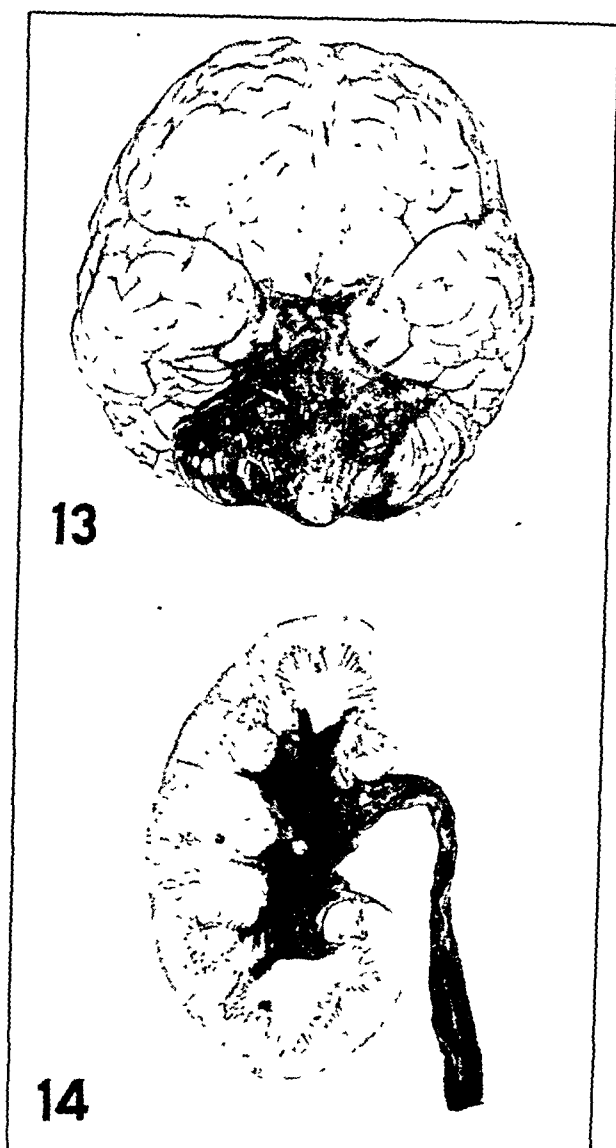


FIG. 13.—Basal hemorrhage originating at an undetermined site in the cerebrum, filling the lateral and third ventricles with blood, rupturing the septum pellucidum, and breaking through into the sub-arachnoid space.

FIG. 14.—Hemorrhage in the renal pelvis extending along the ureter resulted in extensive hematuria, a frequent finding in the series.

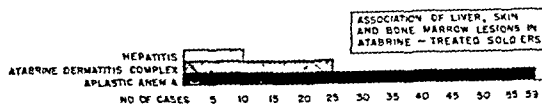


CHART 4

from this Institute by Mullins and Allen, so that these pathologic changes will not be discussed here.

Tissue atabrine values, available in only 1 case, were reported as follows:

Site	Atabrine (mg./kilo)	Atabrine degradation products (mg./kilo)
Liver	16.8	1.7
Spleen	16.2	0
Kidney	6.6	0
Skin, normal, chest	6.6	0
Skin, normal, thigh	12.9	0
Skin, Lesion A	5.9	0
Skin, Lesion B	7.1	0

Bone marrow: 3.5 gamma atabrine extracted from 20 gm. of rib; no degradation products. (This corresponds to 0.175 mg./kilo and is probably not a valid figure.)

This patient took 0.2 gm. of atabrine daily for 10 days beginning May 30, 1944, followed by 0.1 gm. daily until Feb. 28, 1945, 24 days prior to death.

CLINICAL MANIFESTATIONS. The most common complaint of the *prodromal period* was weakness, often associated with headache, vertigo and dyspnea. A hemorrhagic tendency was frequently noted early, evidenced by bleeding gums after brushing teeth, nosebleed, "bruising easily," or the spontaneous appearance of ecchymoses in the skin. Dimness of vision noted first by 2 of the patients was found to be due to intraocular hemorrhage. In the majority of cases the onset was gradual, several weeks to a month or even more elapsing before the men reported to sick call. The prodrome was of brief duration in 6 cases and the onset precipitous in 3 in which an acute febrile state was the first indication of illness.

The early manifestations of aplastic anemia were often unnoticed in the group of 25 patients who were being treated for skin lesions until individuals among them were observed to be rather pallid and a blood count disclosed a reduction in the formed elements of the blood. In no instance did the anemia precede the dermatitis.

The course was almost invariably marked by fever and hemorrhagic phenomena, the location and extent of the hemorrhages accounting for differences in symptoms.

For example, hemiplegia, convulsions, and coma were noted in patients with intracranial bleeding, or hematuria and sometimes flank pain with urinary tract hemorrhage. Four patients were still living at

the time of the last report, 1 having survived 2 months, a second 4 months, and the other two 7 months.

HEMATOLOGIC FINDINGS. Rather complete series of blood counts were submitted in most cases, and the diagnosis of aplastic anemia was confirmed by sternal biopsy in 25.

In 4 cases blood counts were performed very soon after the onset and showed that the red cell level was fairly well maintained (4,000,000 to 5,000,000 per c.mm.), whereas the leukocyte and platelet counts had fallen sharply, the lowest being 1500 and 40,000 per c.mm. respectively. This is to be expected when one recalls the relatively long life of the mature erythrocytes (average 60 days as opposed to 3 to 5 days for neutrophils and platelets), and that most of the residual cells found in the bone marrow at autopsy are their progenitors.

The other patients were already anemic when first examined and initial erythrocyte counts as low as 600,000 per c.mm. were recorded, although the average range was 1,500,000 to 3,000,000 per c.mm. The anemia was generally of the normocytic, normochromic type; in some cases it seemed to be hyperchromic, but this is questionable since it was reported only where anemia was severe, *i. e.*, in the range where hemoglobin readings are apt to be very inaccurate. Furthermore, no hematocrit values were available in these cases to check for macrocytosis. Nucleated red

cells almost never appeared in the peripheral blood. Reticulocyte counts over 1% were rarely recorded and they were frequently reported as either less than 0.1% or "none found." Anisocytosis and poikilocytosis were seldom marked except in the few cases showing evidence of regeneration. It was possible with frequently repeated blood transfusions to restore the erythrocyte and hemoglobin levels to normal or nearly so in 16 cases and to effect an improvement in nearly all. A few patients became progressively more anemic despite transfusions and the majority did not maintain their bettered status.

The initial leukocyte count was seldom over 3000 per c.mm. and usually fell between 1000 and 3000, being normal (7200) in only 1 case. Values below 1000 per c.mm. were noted in 5 instances. The counts fluctuated somewhat, and occasionally rose following transfusions, but the general trend was toward lower levels as the disease progressed, with little tendency to increase significantly even during clinical remissions. The leukopenia was due primarily to failure of the bone marrow to produce granulocytes, but when the total count was greatly reduced the relative lymphocytosis was actually a lymphopenia. This was also the case with respect to the monocytes. Absolute agranulocytosis was frequently recorded, but a general scan of the blood films usually disclosed a few neutrophils, most of them segmented. Immature granulocytes were occasionally seen early in the course, but rarely thereafter.

Blood platelet counts were significantly low in all but 4 of the 49 cases in which they were done. In 3 of these exceptions only 1 platelet count was made and that early in the course of the disease; in the fourth 2 counts were recorded as 263,000 and 154,000 per c.mm. Autopsy disclosed extensive visceral hemorrhages in every one of these cases, however, and the marrows were devoid of megakaryocytes, indicating that subsequent platelet levels must have been much reduced. In general the counts ranged between zero and 100,000

per c.mm., most below 50,000 at some time during the disease.

The coagulation time was little altered, but clot retraction was slow or absent. Bleeding time was sometimes normal even though the tourniquet test was positive; in other cases the bleeding time was prolonged, the lancet prick oozing for several days in 1 instance.

Aspirates of the *sternal bone marrow* were studied in 23 cases and fragments of the bone in 2, the latter showing the characteristics already described. Fat was conspicuous in many of the slides prepared from the aspirated material and the nucleated cell count ranged between 5000 and 10,000 per c.mm. in the few instances in which it was recorded. As one would anticipate, the differential bone marrow cell counts varied considerably with the technique employed and the degree of dilution with peripheral blood. Sample counts are shown (in percentages) in the following tabulation. Cases A and B terminated fatally. In Case C there was promise of recovery, both clinically and from the change in character of the successive marrow biopsies; C (Fig. 9) was taken after 17 days of illness, C' (Fig. 10) after 72 days.

The count in Case A and the first count in Case C would imply either nearly complete aplasia of the marrow or extensive dilution with peripheral blood. The presence of considerable fat in the smears, however, in addition to plasmocytes and hemosiderin-laden histiocytes, signifies that they are representative specimens of the marrow. The second count in Case C shows spontaneous regeneration after the patient had been tided over the aplastic phase with 22 blood transfusions. The marrow aspiration in Case B was performed during the early retrogressive period of the disease and the count reflects some residual hematopoietic activity; the autopsy 1 month later disclosed virtually complete aplasia apart from a few tiny foci of normoblasts. These few but representative examples indicate that biopsy of

the sternal bone marrow is usually of diagnostic and prognostic significance.

Discussion. During a period for which records are reasonably complete (1943 and 1944), nearly 2½ times as many cases of aplastic anemia occurred among troops stationed in the South and Southwest Pacific Areas and the Asiatic Theater combined as in the entire Army elsewhere, even though the troop strength in these regions varied from one-twenty-fifth to one-seventh that of the larger group (Chart 1). Expressed as cases per 100,000, this disproportionate incidence is even more striking and it shows a rapid and steadily progressive increase with each succeeding ½ year period (Chart 2) up to the first half of 1945, the returns from

action. This regime differs radically from that employed in the Mediterranean Theater where the usual dose was 0.3 gm. per week and the supervision sometimes desultory; furthermore, suppressive medication was omitted entirely during the winter.

Thus, apart from geographic location, the single common factor unique to troops in the tropical Pacific and Asiatic Theaters was the protracted use of atabrine. Were it possible to eliminate from consideration the men stationed in non-malarious or sanitized zones within these theaters the incidence of aplastic anemia among those actually taking atabrine would be still higher. Even if the few patients who also receive sulfonamides were excluded, the

	Case A	Case B	Case C	Case C
Myeloblasts	0	2	0	3.5
Neutrophil:				
Myelocytes A	0	4 5	0	15
Myelocytes B	0	7 5	0	10
Myelocytes C	0	5	2 5	7
Stab forms	0	5	1	12
Segmented forms	4	4	2	8
Eosinophils	1	0	0	0
Proerythroblasts	0	9	0	6
Erythroblasts	1	18 5	1	10
Normoblasts	3	7 5	1	12
Megakaryocytes	1	0	0 5	0 5
Lymphocytes	81	30 5	80	11
Plasmocytes	5	3	4	2
Monocytes	1	1	3	1
Histiocytes	3	2 5	5	2

which are incomplete. In contrast, the incidence of aplastic anemia in the Army exclusive of these particular areas remains remarkably constant.

In the malarious areas of the Pacific and Asiatic Theaters 0.1 gm. of atabrine was given 6 or 7 times a week continuously and for at least 4 weeks after redeployment to non-infected zones. Directives called for the drug to be administered by roster to both officers and men, with a competent non-commissioned officer to witness the actual swallowing of the tablets. Although there undoubtedly was a certain amount of laxity in the earlier months, the value of suppressive treatment became so apparent that failure to comply became a matter for disciplinary

incidence curve would remain significantly high. It has been suggested that the surreptitious use of sulfonamides as a venereal prophylactic might have been responsible for some cases, but this practice was unquestionably more prevalent in the European and Mediterranean Theaters than in the Pacific Islands.

It has been fairly well established that the so-called New Guinea lichen planus is caused by atabrine and the condition has recently been designated "atabrine dermatitis complex." It may be of some significance that in 25 of the 57 cases these skin lesions preceded the development of the anemia. Therapeutic agents directed toward the dermatitis were not recognized as likely to produce aplastic anemia except

the single case in which Roentgen ray was used, but here the dosage employed was too small to be regarded as dangerous.

For these reasons atabrine is believed to be the cause of most cases of aplastic anemia occurring among troops receiving drug suppressive treatment continuously for months. The rapid rise in rate per 100,000 over 4 successive $\frac{1}{2}$ year periods is probably a reflection of the length of treatment, perhaps influenced also by an increasingly rigid discipline regarding administration of the drug. The individuals may have slowly acquired hypersensitivity, the antigen possibly being formed through the interaction of the drug or one of its radicles and a protein. The rôle of overdosage cannot be satisfactorily evaluated.

Conclusions. 1. Atabrine administered in suppressive doses over a period of months may cause aplastic anemia.

2. The incidence of aplastic anemia among troops treated continuously with atabrine is far higher than among other soldiers.

3. The actual number of cases of aplastic anemia attributed to the use of atabrine is infinitesimal as contrasted with anticipated morbidity and mortality from malaria had drug suppressive therapy not been employed.

4. The increased rate of aplastic anemia among troops given atabrine in suppressive doses does not constitute a contra-indication to the use of the drug.

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PROGRESS OF MEDICAL SCIENCE

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TEMPORAL ARTERITIS AND LOSS OF VISION

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WHEN Horton, Magath and Brown⁷ first described the syndrome of temporal arteritis in 1932, they regarded it as an essentially localized or isolated arterial lesion, though recognizing that the systemic manifestations were disproportionate to the local arterial lesions. In a further report in 1937, Horton and Magath⁸ emphasized that the subjective symptoms, the clinical findings and the histologic changes in excised portions of the temporal arteries were so typical and so essentially identical in all cases as to constitute a new, or at least previously undescribed clinical entity. Cases described subsequently by other authors confirmed the actuality of this entity but showed also that the involvement of arteries might be more generalized than was suspected originally. In fact, in 1943, Horton¹⁰ stated: "We always have suspected that the arteritis in this condition was more extensive than could be detected on careful physical examination. Recent observations confirm this impression." A recent editorial⁵ recognizes temporal arteritis as a new disease entity. It calls attention to the fact that the

studies of Gilmour,⁶ of Sproul,¹⁵ of Chas-noff and Vorzimer,² and of Cooke and his associates³ have demonstrated that "arteritis of the temporal vessels is probably a local manifestation of a systemic arterial disease which cannot be regarded as non-fatal."

Perhaps the first clinical evidence that the arterial disease in this syndrome was not confined to the temporal vessels was furnished in 1938 by the occurrence of bilateral blindness due to closure of the central arteries of the retinas in a case of temporal arteritis observed by Jennings and Comb.⁹ Horton and Magath⁸ stated in 1937 that evidence of phlebitis of one of the retinal veins with hemorrhages and exudates had been observed in 2 of their cases. It seemed difficult to connect this in any direct way with the arteritis of the temporal vessels. However, the report of Jennings and Comb⁹ was followed by reports of similar cases by Dick and Freeman,⁴ by Scott and Maxwell¹³ and by Johnson, Harley and Horton.¹⁰ Thus it became evident that loss of vision was not a chance accompaniment but a definite part of the syndrome of temporal arter-

itis. It appears that involvement of vision is to be expected in about one-third of patients who have arteritis of the temporal vessels. Johnson, Harley and Horton¹⁰ suggested the possibility of a direct extension of the lesion from the temporal arteries into the arteries of the optic nerves. They stated: "The anterior deep temporal arteries have an anastomosis with the lacrimal branch of the ophthalmic artery. The ophthalmic artery gives off the central retinal artery, which sends an anterior and posterior branch to the optic nerve. It would be conceivable that the inflammatory process in this case had traveled in this direction." In view of the demonstration of lesions in arteries in various parts of the body in this syndrome, demonstration of such direct extension of the process would not seem to be necessary to explain the involvement of the central artery of the retina.

As stated by Cooke and his associates,³ "There exists in elderly people a widespread arterial disease, not uncommon, but rarely recognized, in which characteristic arterial and striking local signs occur, and in which inflammatory and degenerative changes in the walls of the affected arteries produce a characteristic histologic picture." These authors state that 37 cases have been reported, in 18 of which there was some visual disorder. The patients varied in age between 55 and 80; 25 were among women and 12 were men. Some involvement of the extra-ocular muscles has been reported in 8 cases. The loss of vision may occur suddenly or may progress slowly over a period of several weeks. The 2 eyes may be involved simultaneously or a considerable interval of time may elapse between involvement of the first and second eyes. At times, only 1 eye is involved; but in most cases both are affected. The visual defects may vary from mistiness of vision and different patterns of field defects to complete blindness. These authors state that, although there is usually some permanent impairment of vision, some cases with even moderately severe loss for as

long as 5 days may make a fairly good recovery. "The disease process must affect the arterial supply of the optic and oculomotor nerves and the retina in varying degree, sometimes with complete thrombosis of the affected vessels and at other times cutting down the blood supply by temporary swelling of the vessel wall and later allowing eventual restoration of function, providing the degree of ischemia has not been too great." This would seem to be a logical explanation for the great variance in the subjective loss of vision and in the objective findings in the ocular fundi in the reported cases. However, in my experience, there has been little tendency to recovery of lost vision in patients with this disease. As noted by Johnson, Harley and Horton,¹⁰ treatment with vasodilator drugs has proved to be actually harmful rather than helpful.

Cooke and his associates³ have presented the only histopathologic studies to date of the central retinal arteries in a case of temporal arteritis. At the time of death, neither eye had light perception. The optic disks showed pallor and "primary atrophy." At necropsy, the "optic nerves showed marked softening." Sections of the central arteries of the retinas showed diffuse inflammatory changes spreading from the adventitia through the media. The lumina were obliterated by cellular fibrous tissue formed apparently during organization of a thrombus. The adventitia and media were infiltrated by lymphocytes and plasma cells and the internal elastic lamina was fragmented and calcified. A few giant cells were phagocytosing these fragments. The changes could be traced forward into the branches of the arteries.

In many of the reported cases, descriptions of the lesions in the ocular fundi have been rather vague and difficult to classify. This is not due entirely to poor or incomplete observation and recording, as a striking feature of the disease is the fact that the loss of vision in an individual case may be quite out of proportion to the visible changes in the optic disk or retina.

It is possible for sudden complete blindness to occur without visible lesions in the fundus, as in 1 eye of a patient observed by Johnson, Harley and Horton.¹⁰ Such a case must be classified probably as a retrobulbar neuritis due to ischemia or anoxemia. On the basis of reported findings and of my observations in several unreported cases, it would seem logical to classify the lesions seen by ophthalmoscopic examination of patients with temporal arteritis into 3 groups: closures (thromboses) of the central artery of the retina or of branch arterioles, ischemic optic neuritis, and indeterminate. In the last group are included cases in which the findings are too vague to classify but are probably associated directly with the arteritis of the temporal vessels and also cases in which the changes observed in the retina may be purely incidental. Perhaps the most frequent findings is that of ischemic optic neuritis. This would seem to fit in logically with the presumed course of the disease process.

Three cases would seem to fall with fair definiteness into the group of arterial closures. In the case reported by Jennings and Comb,⁹ there was complete obstruction of the central retinal artery of the left eye and "progressive obliteration of the right central retinal artery with special narrowing of its upper branches." In Case 1 of Johnson, Harley and Horton,¹⁰ there were several ischemic areas along the superior temporal vessels of the right retina and occlusion of the upper branches of the left central artery and vein with fragmentation of the blood stream and ischemic edema at the upper pole of the optic disk. Cooke and his associates³ state that in their Case 1 there was a "vascular block" in the left eye. The ophthalmoscopic description is rather atypical, however, since it was stated that the disk was blurred and pale, the arteries of normal size, the veins engorged and compressed, and there was a patch of exudate above the macula.

Seven cases would seem to fall into the group of ischemic optic neuritis. In Dick

and Freeman's⁴ Case 1, the optic disk was twice normal in size and was slightly edematous with a small hemorrhage on its surface. In Johnson, Harley and Horton's¹⁰ Case 2, the right fundus was essentially normal. The left optic disk was mildly edematous and slightly pale, an area of whitish exudate extended out about 1 disk diameter from the temporal margin of the disk, and several small hemorrhages were present in the superior nasal retina. In their Case 3, the right optic disk was pale and slightly edematous nasally and there was an area of localized edema of the retina between the disk and the macula; the left optic disk was edematous and was rather pale in its lower temporal quadrant. In the case reported by Shannon and Solomon,¹⁴ ophthalmoscopic examination revealed edema of the disks and retinas, threadlike arteries, engorged veins and some minute hemorrhages. Three of the cases reported by Cooke and his associates³ seem to fall into this group. In their Case 2, in the right eye the disk was edematous and pale, the arteries were contracted, and there were in the retina 1 hemorrhage and couple of areas of edema or exudate; in the left eye, the disk was edematous and pale. In their Case 3, both optic disks were edematous and pale, the arteries were normal. In their Case 6, the right optic disk was swollen and the left normal; later both optic disks were pale and atrophic.

Nine cases must be placed in the indeterminate group; 4 of these would seem to be associated definitely with the syndrome of temporal arteries. In the case reported by Scott and Maxwell,¹³ at the first observation, the optic disks were normal and the retinal vessels were essentially normal. Two months later, the right iris was atrophic and lens opacities prevented a view of the fundus; numerous small patches of exudate were present in the left peripapillary retina. No explanation was given for the loss of vision in Dick and Freeman's⁴ Case 2 or in the case reported to Horton by R. A. Johnson, Cooke and his associates³ state with refer-

ence to their Case 5 that, in the right eye, the optic disk was normal, the arteries were small but showed little sclerosis, and there were some "whitish subretinal hemorrhages" in the macular region; in the left eye, the optic disk was pale and atrophic with distinct edges, the arteries were very small and the veins large, the macular retina showed some edema.

In the 5 remaining cases in the indeterminate group, the evidence of direct connection with the temporal arteritis is not convincing. Attention has been called previously to the 2 cases of retinal phlebitis noted by Horton and Magath.⁸ In Bain's¹ case, there was said to be severe photophobia and bilateral peripapillary atrophy. In Case 7 of Cooke and his associates,³ the optic disks were normal and the retinal vessels were generally narrowed with slight arteriosclerosis. Post and Sanders¹² reported their ophthalmoscopic observations in a woman, aged 66, who had been seen 6 years before, suffering from typical arteritis of the temporal vessels, by MacDonald and Moser.¹¹ At this time, the ocular fundi were normal. When the patient was seen by Post and Sanders,¹² there was no visual complaint. The optic disks were normal. The arteries were markedly sclerosed and irregular in caliber. In the right retina, there were several areas of periarterial sheathing with apparent fusiform thickening of the wall through which the blood column could

not be seen. Below and nasal to the macula were 3 confluent areas of silvery exudate about 1 disk diameter in extent.

The increasing number of recent case reports would suggest that the entity of temporal arteritis is of more frequent occurrence than had been supposed previously. It is possible that through more general recognition of this syndrome an explanation of sudden complete or partial loss of vision in certain elderly individuals might be found in otherwise unexplained cases. Suspicion of the possible presence of arteritis of the temporal vessels should be entertained especially when the patient's history of the mode of onset of the visual loss suggests a possible closure of the central artery of the retina but the ophthalmoscopically visible lesions are atypical. When the loss of vision occurs during the phase of active involvement of the temporal arteries associated with severe headache and cordlike swelling and tenderness of the arteries and the complaint of pain or fatigue on chewing, the presence of the syndrome is rather readily recognized. At times, however, involvement of the eyes does not occur until several months after the subsidence of the acute phase of temporal artery inflammation. In such instances, a careful taking of the history is necessary to elicit the salient points which will permit of survival at the correct diagnosis.

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PHYSIOLOGY

PITUITARY-DIABETES

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PITUITARY DIABETES is the diabetes which persists after a short course of anterior pituitary extract. It has been divided into the temporary phase in which the hyperglycemia and glycosuria caused by the extract disappear if the extract is discontinued, and the permanent phase which continues after a more prolonged course of extract. The extracts originally used by Johns, O'Mulvenny, Potts and Laughton,²⁷ Houssay,^{23a} and others to produce temporary diabetes and by Evans, Meyer, Simpson and Reichert,¹⁴ and Young^{50a} to make dogs permanently diabetic were crude saline extracts of the anterior lobe of beef pituitaries. Since these early studies, many investigations have added to our understanding of this form of diabetes. The recent monograph by Soskin and Levine⁴⁷ contains a valuable summary of pituitary-diabetes.

Today the 3 aspects of pituitary-diabetes which appear to deserve particular consideration are: (A) the identification of the hormones concerned with the metabolic activity of the pituitary; (B) the physiologic mechanism by which pituitary extracts, crude or refined, initiate the phenomena of diabetes; (C) the pathogenesis of the permanent phase of diabetes with its obvious lesions of the islands of Langerhans must be related to the preceding functional effects of the pituitary extract. The various aspects of pituitary-diabetes have been so well reviewed that it is only necessary to survey the present state of affairs.

A. PITUITARY HORMONES. Fraenkel-Conrat¹⁶ recently reviewed the chemistry of the pituitary hormones, and Jensen²⁶ has kept the subject up-to-date. By means of the ultra-centrifuge, electropho-

resis and other modern methods of protein chemistry, 5 major hormones of the anterior pituitary have been obtained in pure form. The sixth, growth hormone, has been tested in a state where only 2 proteins are present. Although a single protein with growth activity has recently been isolated by Li, Evans and Simpson,³⁰ its metabolic effects have not been determined. The thyrotrophic, lactogenic and 2 gonadotrophic hormones have been obtained as single proteins. In this state they manifest their respective activities with practically no overlapping of other pituitary functions. As a result, it is possible to exclude them from any major part in the diabetogenic activity of the pituitary. The exclusion of these purified hormones is in accord with Young's^{50b} conclusion that the gonadotrophic and thyrotrophic substances were not diabetogenic *per se* in the dog. This means that the growth and adrenotrophic hormones are the elements largely responsible for the metabolic effects under discussion. They have been isolated so recently that extensive studies are not yet available, but Table 1 outlines the present knowledge of their action on metabolism.

The promotion of growth by growth hormone and its inhibition by adrenotrophic hormone (Table 1) have been measured by their effects on gain in weight and on epiphyseal proliferation in hypophysectomized rats,^{15,38} and the authors have pointed out the antagonism between these pituitary fractions.

The lowering of the respiratory quotient by growth hormone has been reported by Greaves, Freiberg and Johns²⁰ and Fraenkel-Conrat.¹⁶ No direct studies of adrenotrophic hormone on the R.Q. have been

made but Greaves *et al.* noted that a fraction potent in lowering the R.Q. had no trophic action on the adrenals. Further indirect evidence is derived from the study of adrenal cortical extract and its metabolically active steroids. Long, Katzin and Fry³² found that the adrenal hormones depressed the R.Q. of glucose fed rats. Thorn *et al.*⁴⁹ observed this in patients with Addison's disease. Russell^{42a} compared the effects of crude pituitary extract and adrenal cortical extract. She found that although both depressed the R.Q. they were quite distinct in their action on glycogen. Cortical hormone increased liver glycogen but did not directly affect muscle glycogen, whereas APE maintained muscle glycogen and did so independently of the adrenal cortex.

therefore, listed as doubtful. Although both growth and adrenotrophic fractions may lower the R.Q. the evidence indicates that they do so in different ways, and this probable common effect does not prevent one from distinguishing their physiologic identity.

The ketogenic activity of crude pituitary extracts observed by Burn and Ling⁷ has since then become closely associated with the growth activity. This was studied by Greaves *et al.*²⁰ who also noted the close correlation between the lowering of the R.Q. and ketogenic activity. The fact that the purified growth hormone, described herein, contains 2 substances¹⁶ and the findings of Shipley,⁴³ and Shipley and Seymour⁴⁵ that growth and ketogenic activity are due to 2 separate principles

TABLE 1.—COMPARISON OF THE METABOLIC EFFECTS OF PURIFIED GROWTH AND ADRENOTROPHIC HORMONES

Growth	Adrenotrophic
1. Promotes growth	1. Inhibits growth
2. Lowers R.Q.	2. Effect on R.Q. doubtful
3. Ketogenic	3. Not ketogenic (inferred)
4. Nitrogen retained	4. Nitrogen unchanged or lost
5. Lowers blood amino acids	5. Amino acids unchanged
6. B.U.N. and N.P.N. unchanged	6. Increases B.U.N. and N.P.N.
7. Liver arginase unchanged	7. Increases liver arginase
8. Maintains muscle glycogen (short experiments)	8. Maintains muscle glycogen (long experiments)
9. Lowers pancreatic insulin	9. Increases pancreatic insulin
10. Slightly increases glycosuria of depan. rats	10. Markedly increases glycosuria of depan. rats

The suggestion that these substances differ in their mode of action has been supported by Bennett and Perkins,⁴ and by Russell's subsequent work.^{42b} Here, likewise, the glucose requirement of eviscerated hypophysectomized rats which is greater than normal was reduced by both pituitary and adrenal hormones, but the concomitant changes in glycogen led to the conclusion that the pituitary and adrenal hormones affect carbohydrate metabolism by different means.^{42b} She noted that the R.Q. was depressed only in rats fed large amounts of glucose. One should add that the steroids surviving extraction or those given in pure form may not be just the same as the secretion induced by the adrenotrophic hormone. The effect of adrenotrophic hormone on the R.Q. is,

of similar molecular size, should pave the way for further identification. No studies on the ketogenic action of the most purified growth preparations are at hand.

The ketogenic effect of pure adrenotrophic hormone is also unreported. The assumption that it is not ketogenic (Table 1) is based on the finding of Shipley and Fry,⁴⁴ that adrenal cortical compounds do not stimulate ketosis.

The well-known ability of pituitary extract to promote nitrogen retention has been identified particularly with the growth hormone by Marx, Magy, Simpson and Evans.³⁷ They did not use purified adrenotrophic hormone but adrenal cortical extract had no such effect. In other investigations, summarized by Long, Katzin and Fry,³² nitrogen excretion was

increased by the adrenal cortex and the contrast expressed in Table 1 seems to be a logical assumption. The different effects of these pituitary principles on nitrogen metabolism receives further support from the study of their action on the nitrogenous elements of the blood. Fraenkel-Conrat, Fraenkel-Conrat and Evans¹⁷ reported the changes listed in Items 5 and 6 of Table 1, using the purified pituitary fractions. In addition, Fraenkel-Conrat, Simpson and Evans¹⁹ distinguished these principles by their action on liver arginase.

The relationship of the pure principles to muscle glycogen has been measured by Herring and Evans.²² Although both maintain muscle glycogen, they do so under different conditions. These results supplement the studies of Russell^{42a,b} already cited and all investigations are in agreement with the concept that the growth and adrenotrophic effects influence carbohydrate metabolism by different mechanisms.

The effect of pituitary hormones on the insulin content of the pancreas has been measured by Fraenkel-Conrat, Herring, Simpson and Evans.^{18a,b} This effect may well be secondary to the alteration of the metabolic constituents because it is comparable to the changes induced by diet.³

Finally, the influence of growth hormone on the glycosuria of partially depancreatized rats has been recorded by Marx, Anderson, Fong and Evans.³⁶ They found that there was a definitely increased glycosuria in 5 of 13 rats tested, the other animals having only slight changes. Compared to this slight response the powerful glycosuric action of adrenal cortical compounds³² and the glycosuric action of crude adrenotrophic preparations provides, in the Reviewer's opinion, a contrast between these hormones.

Summarizing this section, the tentative conclusion is that when purified hormones are used there is a good physiologic distinction between the 2 fractions which are responsible for most, if not all, of the diabetogenic activity of the whole gland. The extent to which 1 of these principles

is diabetogenic cannot be determined until larger quantities of the pure hormones are available for use in dogs. The absence of hyperglycemia from the activities listed in Table 1 is noteworthy. This action of pure growth hormone is not recorded, but Long, Katzin and Fry³² reported an increase in blood sugar from 114 to 154 mg. per 100 cc. when rats fed glucose were treated with cortical extract. Still more striking was Ingle's^{25a} demonstration that 11 dehydro - 17 - hydroxycorticosterone caused severe hyperglycemia and glycosuria in normal, fed rats. More recently Ingle, Winter, Li and Evans^{25b} have produced glycosuria in normal rats by pure adrenotrophic hormone. At present one can only admit that all diabetogenic activity has not yet been identified with a single hormone, although the means of doing so appear to be at hand.

There is no quantitative assay for diabetogenic activity and possible differences in the methods used must be kept in mind. Thus, a rat deprived of 95% of its pancreas may be so sensitive that an increase in glycosuria may result from small doses of growth or adrenotrophic hormones, and from what has been said these results might be due to either underutilization or overproduction of carbohydrate. Such sensitive animals are suitable preparations with which to eliminate the clearly inactive fractions. On the other hand, a much more potent hormone is needed to produce diabetes in the normal dog and there is no evidence that any pure hormone will do this. In addition, the numerous functions which may share in the diabetogenic effect demand multiple assay methods. In Table 1, Items 2, 3 and 10 are diabetogenic properties of the growth hormone and most of the effects of adrenotrophic principle are appropriate to a diabetogenic hormone. Therefore, several of the present methods of assay will be required to elucidate the rôle of the pituitary principles concerned with metabolism.

B. DIABETOGENIC MECHANISMS. Pending further investigation of purified ex-

tracts it is well to reëxamine the action of crude extracts in order to appraise the physiologic mechanism by which the anterior pituitary is diabetogenic. For the purpose of discussion, it will be assumed that the hormones act for the welfare of the peripheral tissues, and hence the action of the anterior pituitary will be reviewed with respect to its action on muscle. The control of muscle glycogen will be the focal point to which the other effects of the gland will be referred. The influence of the hormones on blood sugar, liver glycogen, and ketogenesis will be briefly related to the changes occurring in the muscles.

when the various studies^{24,31,42a,b,50b} are reviewed it seems clear that the anterior pituitary maintains glycogen or permits its increase. This does not imply that it promotes glycogen deposition by the same mechanism or to the same degree as insulin. Both insulin and pituitary extract can increase the glycogen level but this increase is always accomplished in the presence of both hormones. In such a system, either insulin or the anterior pituitary may be in excess, but the hormone which is relatively inactive still exerts some influence. This is no new observation. For example, Soskin⁴⁶ notes that insulin is "but one factor in a bal-

TABLE 2.—SOME EFFECTS OF INSULIN AND ANTERIOR PITUITARY EXTRACT (APE) ON MUSCLE GLYCOGEN

Procedure	Hormones present	Muscle glycogen	Other effects				Schematic effect	
			Liver glycogen	Blood sugar	Protein to COH	Keto-genesis	Glucose to cell	Glucose from cell
Glucose to normal	Insulin	+	+	+	—	—	++	—
	APE	+	—	—	—	—	++	—
Insulin to normal	Insulin	+	—	—	+	+	—	—
	APE	+	+	+	+	+	—	—
APE to normal	Insulin	+	+	++	++	++	—	—
	APE	+	—	++	++	++	+	++
Pancreatectomy	APE	—	—	—	—	—	—	++
Hypophysectomy	Insulin	—	—	+	—	—	—	++
Houssay animal	"None"	—	—	+	—	—	—	++

+ = increased; — = decreased.

Table 2 shows the effects on muscle glycogen of the administration and of the removal of insulin and the anterior pituitary. The response to glucose needs no comment and the action of insulin on muscle glycogen is well known. The effect of anterior pituitary extract on muscle glycogen has been studied by Houssay, Biasotti and Dambrosi,²⁴ Young,^{50b} and Long.³¹ The data of Long on the increased muscle glycogen in mice when glucose and pituitary extract were administered have been compared with the increment found by Bridge⁶ when glucose and insulin were given. It is true that in some experiments the administration of pituitary extract has been associated with a fall in muscle glycogen. However,

anced endocrine system," and Young^{50c} cites the complex action of growth hormone as an "example of the duality of many biologic systems which may be constituted from two or more balanced reactions." The antagonism between the anterior pituitary and insulin has often been outlined and is in part shown in Table 2 in the column listing "other effects." However, the fact that the opposing hormones act together to produce a common effect on the muscle has received less attention than the contrast between the hormones. One may postulate that the primary purpose of insulin and the anterior pituitary is to maintain the "nutrition" of the muscles (tissues) at all times. The action of insulin seems to

dominate when the animal is fed; the action of the anterior pituitary is essential during fasting.

The last 3 lines of Table 2 show that muscle glycogen is lowered when one or both hormones are removed. After hypophysectomy muscle glycogen falls rapidly during fasting although the fed hypophysectomized animal maintains its muscle glycogen fairly well.^{23b,24} The fall in muscle glycogen in depancreatized animals is slight,^{10,33a} as is the case in the more variable glycogen levels of the Housay animal.

The list of other effects (Table 2) could be amplified but the brief data given show the variety of adjustments in the metabolism of protein, fat and carbohydrate which crude pituitary extract may make, largely due to the intervention of the liver. The + and - signs indicate tendencies to increase or decrease which may be more or less apparent according to the experimental conditions. Glycogen levels depend on the availability of glucose as well as on the action of the hormones. The ingestion of glucose, the mobilization of glucose from endogenous sources and, in the absence of insulin, the high level of blood sugar are recognized factors in the formation of muscle glycogen which in the table has been related only to hormones. In this connection the low glycogen levels of the phlorhizinized animal³⁹ in which both hormones are present, and the lower glycogen in fasted hypophysectomized animals than in Housay animals with hyperglycemia, are examples of the rôle played by the blood sugar level in the maintenance of muscle glycogen.

The last column of Table 2 attempts a schematic explanation of the changes in muscle glycogen under the various conditions. In the normal animal, an increased muscle glycogen is a common effect of insulin and of anterior pituitary extract. At the same time, as indicated under other effects, blood sugar, liver glycogen, gluconeogenesis and ketogenesis are influenced in an opposite manner by these

hormones. Under these circumstances the Reviewer assumed, quite arbitrarily, that the hormones might act on or in the muscle cells. In this tissue, glycogen storage would logically result from a positive or favorable balance between the supply and utilization of glucose within the cell. This concept permits an explanation of the effects of insulin and the anterior pituitary on muscle glycogen under the conditions outlined. One may cite the overworked analogy of the reservoir and suppose that insulin opens the inlet and that pituitary extract acts to close the outflow. (In such a scheme the liver would be an emergency reservoir just up stream.) The possible combinations maintaining a high level in the "muscle reservoir" have been expressed by arrows and + or - signs. Plus signs indicate an acceleration, minus signs a retardation of the reactions indicated by the headings. It is necessary to recall the danger of such simple analogies and to remember that the transfer of glucose to and from the cell may result from the passage of glucose from the capillaries, or through the cell wall, or its utilization in any of the numerous chemical reactions concerned with the storage or oxidation of glucose in the cell. Finally, the primary assumption that these hormones act on the tissues will be questioned by those who regard changes in glycogen as automatic responses to the availability of glucose which is provided and regulated by the liver *via* the blood. The fact that insulin and pituitary extract appear to act quite differently on the phenomena mediated by the liver suggests that their action on the muscles is peripheral. This does not deny the importance of a supply of glucose in these reactions as has been mentioned.

The preliminary report of Price, Cori and Colowick⁴⁰ on the action of anterior pituitary extract and insulin *in vitro* forecasts a new understanding of this whole subject. They studied the reaction: glucose + adenosine triphosphate \rightarrow glucose-6-phosphate + adenosine diphosphate

which is catalyzed by hexokinase. They found that this reaction is inhibited by an anterior pituitary extract containing growth and ketogenic activity.²⁰ Insulin releases hexokinase from the inhibition of the anterior pituitary although insulin by itself has not been observed to alter hexokinase activity. Because the formation of glucose-6-phosphate is a reaction common to the oxidation of glucose and to the formation of glycogen these results support the physiologic estimation that pituitary extract inhibits glucose oxidation. The location of the pituitary extract *versus* insulin antagonism at a single chemical reaction would seem to nullify the "explanation" in the last column of Table 2. Nevertheless, the common action of insulin and the anterior pituitary on glycogen levels in the intact animal, indicated in Table 2, appear to require for their explanation more than is now known of the hexokinase reaction. In like manner the insulin sensitivity of the hypophysectomized animal is not accounted for by an action of insulin exerted only in the presence of the anterior pituitary. It is too early to evaluate fully the significance of these important observations.

Summarizing this section, emphasis has been placed upon the effect of the anterior pituitary in a hormonal system rather than its isolated action. The possible influence of hormones on the tissues, rather than their action on supply depots and the liver, has been assumed. Although this outline requires correction and enlargement it has been offered as an introduction to the conditions under which pituitary extract produces diabetes.

It is now agreed that the anterior pituitary is a factor in maintaining the organism during fasting. For this purpose it achieves a fairly definite quality and quantity of the metabolic mixture. The administration of pituitary extract to fasted animals adds but little to the effect of starvation and does not produce diabetes.^{23a} However, it accelerates the adjustment to fasting so that the impaired sugar tolerance which results in 2 to 3 weeks of

fasting is accomplished in 2 to 3 days by pituitary extract, and it prevents the usual readjustment made by the fasting animal when carbohydrate is fed. Dohan and Lukens (unpublished) have given pituitary extract to fasting animals which were partially depancreatized and in only 1 or 2 with very small pancreatic remnants was a slight glycosuria produced. In the fed animal, either intact dog or partially depancreatized cat, these extracts readily produced diabetes. Under these circumstances it seems that large doses of pituitary extract may fix or set the metabolism in the pattern needed for fasting. When such a low carbohydrate régime is made "rigid" by extract, most of the carbohydrate food ingested is in excess of the amount which can be utilized, so that hyperglycemia and glycosuria result. In the early stage of extract treatment and lacking knowledge of the animals' endogenous metabolism, the factor of overproduction seems to be attributable to food intake rather than to the quantity mobilized by the pituitary from body depots.

Dohan, Fish and Lukens¹¹ have observed the nitrogen retention of crude pituitary extracts prior to the development of severe glycosuria. From these experiments, the data in Table 3 are presented to show that nitrogen retention occurs in the first 3 days of pituitary treatment when hyperglycemia develops, but before the islands are visibly injured. This action of diabetogenic extracts on nitrogen metabolism has also been shown in rats by Cuthbertson, Webster and Young.⁹ According to Lee and Schaffer,²⁹ and Lee²³ the gain in weight during APE is due to nitrogen and water retention and the body fat remains constant or diminishes somewhat. Although protein food is part of the diabetogenic régime, the retention of nitrogen suggests that overproduction of glucose from protein is not unrestricted and probably is not the sole factor accounting for glycosuria. The prevention of hyperglycemia by an isocaloric high fat diet suggests that fat is not a source

of glucose under these conditions. It is, therefore, the reviewer's opinion that pituitary extract (growth, ketogenic or both) suppresses carbohydrate oxidation and that this action is one essential of its diabetogenic effect. The other essential is food from which glucose is produced at a rate for which the adrenotrophic hormone is apparently necessary.

At present, the studies with pure pituitary hormones and those with crude extracts are in fair agreement. They all indicate that the diabetes produced by crude anterior pituitary extracts is the result of an inhibition of the utilization of carbohydrate and of its simultaneous mobilization from food plus endogenous sources. The conclusion that 2 principles—the growth and adrenotrophic hormones—are necessary to diabetogenic action remains valid until more knowledge of the pure hormones is available.

persisted from 4 to 6 weeks in the dog, the island lesions which began as hydropic degeneration progressed to atrophy. On the other hand, partial pancreatectomy unaccompanied by diabetes was not harmful to the islands. The relationship between pancreatic deficiency and diabetes, and the association of diabetes and injury of the islands of Langerhans was recognized before the discovery of insulin.

In like manner, diabetes has been produced by anterior pituitary extract only when sustained hyperglycemia has been effected. The following observations illustrate this point. Some species, such as the mouse, rat and guinea pig, are quite insensitive and do not respond to pituitary extract with hyperglycemia and glycosuria. These animals do not develop lesions of the islands. Moreover, in those species which are susceptible there are individual animals which become refrac-

TABLE 3.—CHANGES IN WEIGHT AND NITROGEN EXCRETION AT ONSET OF PITUITARY-DIABETES

Dog No.	Control—3 day periods			APE—First 3 days		
	Initial weight (kg.)	Gain or loss in weight (kg.)	Average urine N (gm./day)	Average urine		Gain in weight (kg.)
				Nitrogen (gm./day)	Glucose (gm./day)	
P-16	8 35	+0 15	15 67	11.53	2.7	0 40
P-19	5 85	—0 20	14 90	9 80	3.7	0 35
P-22	8 80	—0 10	13 30	9 40	18 8	0 25
P-14	10 45	+0 80	23.60	18 10	1 1	0 85
P-13	16 80	—1 10	6 63	7 60	0	—0 46

The diets varied but for each animal the diet was the same in the control and extract periods. Dog P-13 was fasted for both periods.

This leads to the next question: What is the mechanism by which the lesions of the islands and the permanent phase of diabetes is produced?

C. THE PATHOGENESIS OF THE PERMANENT PHASE OF PITUITARY-DIABETES. Long before the era of pituitary-diabetes it had been ascertained that partial pancreatectomy, if sufficiently extensive, was accompanied by diabetes. Begun by Minkowski and culminating in the work of F. M. Allen, these investigations showed that partial pancreatectomy did not cause lesions of the islands of Langerhans except when hyperglycemia had been present. If this type of diabetes

tory to pituitary extract. After the hyperglycemia caused at first by the extract has subsided, these refractory animals show no island lesions even though the administration of extract is continued. A third point, already mentioned is that pituitary extract is ineffective in fasted, fat-fed, or insulin-treated animals.²¹ In all of these conditions which prevent the development of hyperglycemia there is no damage to the pancreatic islands.

The prevention of diabetes in the presence of an effective diabetogenic procedure deserves further comment. Allen^{1a} showed that "partial pancreatectomy to the extent sufficient for severe diabetes is

followed by no hydropic degeneration when the diabetes is thoroughly controlled by diet." Bell, Best and Haist² have shown that the content of insulin per gram remained normal in pancreatic remnants when diabetes was absent but that it was reduced in similar remnants of diabetic animals. Lukens and Dohan²⁴ have confirmed the prevention of pituitary-diabetes²¹ by the use of isocaloric combinations of meat and fat in partially depancreatized cats. This is recalled not only in connection with the rôle of hyperglycemia in pathogenesis, but to emphasize the need for protein or carbohydrate food in conjunction with pituitary extract, if hyperglycemia is to be maintained. Finally, the occurrence of island damage by pituitary extract has been avoided by the

too small to maintain normal metabolism. Their only animal with atrophy of the islands failed to improve under insulin treatment.

In dogs the characteristic lesion of the islands of Langerhans in the permanent phase of pituitary-diabetes is atrophy.^{11,41} This is so because it takes 4 to 6 weeks of injection to establish permanent diabetes and, as Allen¹² showed, the early hydropic degeneration is followed by atrophy within this period. Lukens and Dohan²⁴ used partially depancreatized cats without spontaneous diabetes in studying pituitary-diabetes. As in the dog, permanent diabetes could be produced by 4 to 6 weeks of extract treatment. Unlike the dog, hydropic degeneration of the beta cells lasted for the

TABLE 4.—PITUITARY-DIABETES IN THE CAT
Treatment Begun Within 3 Months
Effects of therapy

No. expts.	Therapeutic measure	Effects of therapy				
		During treatment		After treatment		
2	200 gm. beef reduced to 100 gm. daily	Becomes normal	Decreased	Largely restored	Recovered	Increased
9	Insulin		Increased		Recovered	
1	Adrenalectomy		Increased		Improved?	
8	Phlorbizin		Unchanged		Recovered	

simultaneous administration of phlorhizin.³⁵ Phlorhizin given at the same time as pituitary extract abolished the hyperglycemia, although glycosuria and ketonuria persisted. Biopsies showed normal islands when the blood sugar was normal and marked hydropic degeneration after the period of hyperglycemia.

Having mentioned the factors involved in the production and prevention of experimental injury to the islands the parallel studies on the recovery of island damage may be reviewed. Soon after the discovery of insulin, Copp and Barclay,⁸ and Bowie⁵ observed morphologic recovery of hydropic islands in partially depancreatized dogs treated with insulin. In spite of the restoration of the islands, recovery of the animals was impossible because the pancreatic remnants had been originally

first 3 months of glycosuria. After 3 months of diabetes, atrophy of the islands ensued as in the dog. Thus, the cat provided a preparation having a stable diabetes with a reversible type of lesion for 1 to 2 months after the cessation of pituitary extract. Within the first 3 months of the disease cats with pituitary-diabetes have been treated, and the islands restored, by the 4 procedures which Table 4 outlines. The anatomic repair of the islands and the functional recovery of the animals have been reported^{12,26,34,35} and discussed. In addition, the whole subject of the pathology of experimental diabetes has been fully reviewed by Duff.¹³ The anatomic recovery following dietary and insulin treatment is quite in line with the earlier observations in partial pancreatectomy. However, as our animals

were not diabetic prior to pituitary treatment the restoration of their originally adequate insular tissue was accompanied by physiologic recovery from the diabetes. Recovery after insulin therapy was achieved in animals with severe diabetes in which 90% of the available glucose of the diet was excreted in the urine. Recovery by means of reduction in diet was only possible when the diabetes was mild and could be controlled by diet. Phlorhizin was effective in severe diabetes and deserves some comment.

zinc impairs the reabsorption of glucose by the kidney; insulin has no such action. Phlorhizin increases protein catabolism as measured by nitrogen excretion; it increases ketogenesis and decreases the amount of food assimilated, at least by mildly diabetic animals. In diabetes, insulin has the opposite effect on these processes. The less certain results of phlorhizin in decreasing carbohydrate oxidation and lowering glycogen stores are probably secondary to the loss of glucose. They differ from the conspicuous and

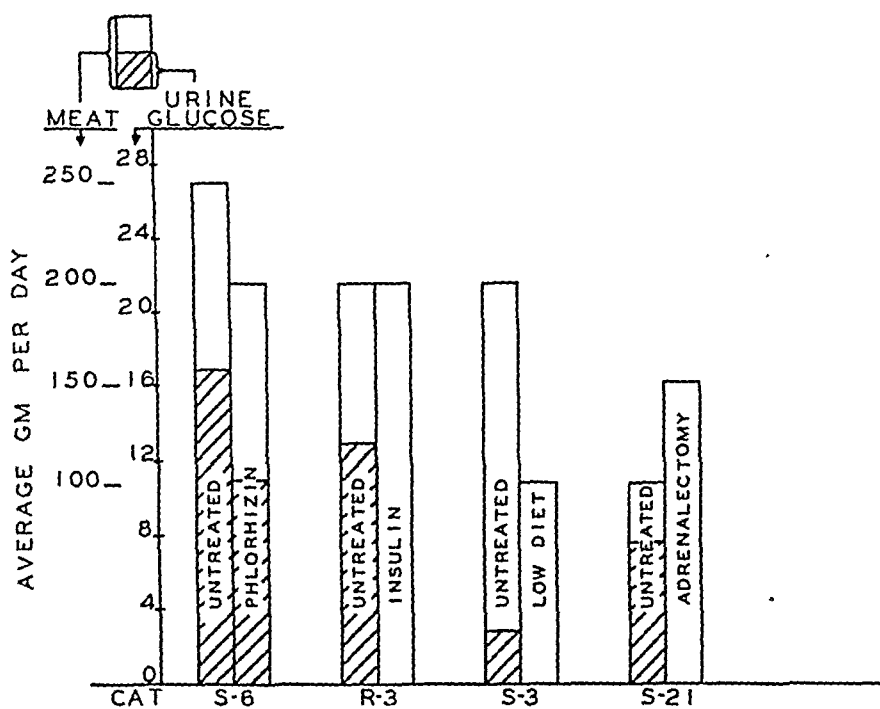


FIG. 1.—Average meat ingested and glucose excreted in 4 types of treatment of early pituitary-diabetes. The daily averages were calculated from 6 day periods before treatment and after treatment had been established. The scales have been so arranged that grams of urinary glucose also represent the available glucose of the diet ($D:N = 3.65$). Thus, the height of the columns, above the hatched area indicating urinary glucose, shows the "glucose retained." The subsequent recovery of the islands has been described (text).

Allen^{1b} and others have demonstrated the ability of phlorhizin to lower the blood sugar level in diabetes. A comparison of the actions of phlorhizin and insulin has been made³⁵ which may be summarized as follows. Each drug lowers the diabetic blood sugar and each one brings about recovery of the islands when the lesions are reversible. Except for these 2 similarities the comparison reveals only contrasts between insulin and phlorhizin. Phlorhi-

probably primary action of insulin on the oxidation and storage of glucose. Admitting the controversial elements which pervade such a summary, the contrast between phlorhizin and insulin, except for their effect on the diabetic blood sugar levels adds further evidence that the normal level of blood glucose is responsible for the recovery of the islands of Langerhans.

Returning to another aspect of the 4

therapeutic measures presented, Figure 1 shows examples of the effect of each procedure on the amount of food assimilated. The scales for the amount of meat eaten and for urinary glucose have been so arranged that grams of glucose correspond to the available glucose of the meat diet when a D:N ratio of 3.65 is used. The total height of the column, therefore, gives the conventional available glucose and the open portion the glucose retained. The writer shares the opinion that the D:N ratio is a constant arbitrarily selected from variable observations.⁴⁶ For this reason it may be well to make the comparison between food intake and urine glucose without making assumptions about the interconversions of the foodstuffs.

are somewhat variable in cats but they correspond approximately to the results obtained in remissions of diabetes in man.^{33a} The conclusion seems to be that recovery is somewhat limited, and this is confirmed by another type of experiment. In 5 recovered animals, diabetes was induced a second time by pituitary extract. Table 5 shows that much less pituitary extract was needed to make these cats diabetic again. It is unlikely that the loss of pancreatic tissue from biopsy or scarring was enough to account for this. The suggestion is made that little more than minimal essential function is regained under treatment so that these islands readily succumb to another stress. These results remind one of the frequency with

Cat No.	PERIOD OF FIRST AND SECOND INDUCTION OF DIABETES		Dosage of anterior pituitary glands	
	Period of pituitary treatment		Diabetes	
	I (days)	II (days)	I (gm.)	II (gm.)
R-10	12	12	60	20
R-12	13	6	33	15
R-21	24	13	36	18
T-7	22	30	42	33
T-8	—	10	50	10
Average	19	14	44	19

The point is that the amount of food assimilated may be increased (R-3, S-21), decreased (S-3) or unchanged (S-6) by the various types of treatment which ultimately lead to the restoration of the islands of Langerhans. It is necessary to emphasize the fact that the responses during treatment, shown in Figure 1 were followed by the full utilization of the usual diet without diabetes after treatment was stopped, as has been shown in the original reports.^{34,35}

The criteria of recovery in these experiments have been freedom from glycosuria, a normal level of blood sugar and the ability to gain weight on the diet taken prior to treatment. In addition, a few animals have had sugar tolerance tests (1.8 gm. glucose per kg. orally). Of 7 cats tested after recovery, 5 had normal and 2 had mildly diabetic curves. These tests

which infection causes exacerbations of clinical diabetes.

From all this it is clear that both partial pancreatectomy and anterior pituitary extract have caused lesions of the pancreatic islands only in the presence of hyperglycemia. Both procedures lead to hydropic degeneration of the beta cells after 1 to 2 weeks of hyperglycemia. If treated early, the islands of Langerhans are restored by 4 measures (low diet insulin, adrenalectomy and phlorhizin) which lower the blood sugar to normal levels. The contrast between phlorhizin and insulin, except for their effect on the blood sugar, has provided further support for the hypothesis that hyperglycemia plays a part in the pathogenesis of diabetes. The preliminary nature of this hypothesis is apparent when one surveys a few of the facts which do not seem to

conform to it. (a) Hydropic degeneration does not occur in the diabetic rat and more species should be studied before we finally interpret the results which appear so clear in the cat and dog. (b) Mild diabetes may persist for long periods in animals and man without rapid progression in the severity of the disease. This stability of mild diabetes is in contrast to the severe glycosuria with a similar hyperglycemia produced by pituitary treatment. (However, in mild diabetes the early island lesions progress to irreversibility as was noted.) (c) Two case reports, 1 by Sprague, Priestley and Dockerty,⁴⁸ and 1 by Duncan, Semans and Howard,¹² compel a cautious attitude toward the rôle of hyperglycemia. In both cases diabetes mellitus of 3 years duration was followed by complete recovery after the removal of adrenal tumors. By comparison with the animal experiments, these patients ought to have had irreversible damage of the islands.

After weighing the pros and cons the bulk of the evidence seems to favor the hypothesis that the level of blood glucose is a factor of importance in the pathogenesis of diabetes. It may well be that hyperglycemia has to be associated with some other factor to produce island damage. If this be so, present knowledge suggests that hyperglycemia may help us to trace the unknown elements in pathogenesis much as the chemist uses a labelled atom to trace substances in the body.

Summary. According to studies done so far, no single purified pituitary hormone contains all the diabetogenic properties. Growth hormone is ketogenic and by the present limited criteria inhibits the utilization of carbohydrate. The adrenotrophic hormone mobilizes protein and carbohydrate and so is necessary for the phenomena of overproduction observed in diabetes. On theoretical grounds, maximum diabetogenic activity would result from a combination of these hormones, and this seems to be the case when crude extracts are employed. The mechanism of action of crude pituitary extracts is still poorly understood. However, this action includes evidence of the suppression of carbohydrate utilization and of overproduction under certain conditions. The effects of crude extract on glycogen storage, protein metabolism and fat metabolism compose a picture not incompatible with the gross observation that protein or carbohydrate food is necessary for the greatest diabetogenic effect. The suggestion has been made that the degree of glucose production and utilization needed by the fasting animal is so established or "fixed" by pituitary extract that carbohydrate or protein food cannot be metabolized in the normal manner and hence leads to hyperglycemia.

If this hyperglycemia is sustained long enough, at least in the dog and cat, the islands of Langerhans undergo a sequence of structural changes resulting in irreversible damage and permanent diabetes.

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SURGERY

UNDER THE CHARGE OF

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ORGANIZING HEMOTHORAX—A CLINICAL ENTITY

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1. **Introduction.** The significance of organizing hemothorax has only recently been recognized and the methods of diagnosis and treatment are largely developments of the recently terminated World War II. That the condition not infrequently existed unrecognized prior to this time can scarcely be doubted. Personal communications from both military and civilian pathologists and others who have had occasion to deal with traumatic disabilities of the chest confirm the fact that the condition was not uncommon, but its nature and clinical significance was entirely missed or imperfectly understood. Seen at operation or autopsy in the years prior to its elucidation, organizing hemothorax was usually misdiagnosed as organizing empyema or simple pleural thickening and so treated and summarily dismissed. The reason for this oversight lies in part with the frequently repeated statement in surgical texts that blood in the pleural cavity clots but rarely, and therefore the basic factor in the development of organizing hemothorax, that is, the presence of clot in the pleural cavity as the underlying pathologic cause of the condition received little attention. With the high incidence of penetrating wounds of the thorax in World War II, however, together with the material advances in the technique concerned with thoracic surgery, the existence of the condition as a more or less definite clinical entity was established and methods for its diagnosis and treatment progressively developed.

Much concerning this disability remains to be clarified, but, as previously stated, the basic principles in its recognition and therapy have already been more or less established. To be convinced of the relative newness of the subject, it is only necessary to search the surgical literature for data concerning it. Such material is extremely scanty and that now available is largely derived from personal communications. The literature concerned with the condition will doubtless continue to grow from both civilian and military sources because of its importance in the special field of thoracic surgery.

There can be little doubt that the condition of organizing hemothorax is of considerable import clinically. Its recognition and successful treatment in World War II has resulted in the restoration to full active military or civilian life hundreds of individuals who would otherwise have remained permanently incapacitated, or at best in a state of restricted activity. To what extent this lesion contributed to the number of those with permanent pulmonary disability during and after World War I is a matter of conjecture, since statistics dealing with the same are not available, but the probability is that it was not a negligible factor.

2. **Definition.** Organizing hemothorax may be defined as a condition usually produced by trauma to the thoracic cavity or its contents in which there exists within the pleural space a clot formed by the resultant hemorrhage which subsequently

undergoes progressive organization by the ingrowth of fibrous connective tissue from the adjacent pleural structures.

3. Incidence. Organizing hemothorax can occur at any age, but since it is practically always produced by trauma to the thorax or its contents, its incidence is far greater in the age groups at which trauma is the most common or during the period of greatest physical activity. It is therefore most common between the ages of 20 and 40. In civilian as well as military life its incidence is far greater in males than in females, and it is far more prone to occur in individuals in the lower strata of society, since strife leading to violence is far more frequent in lower social scales.

The highest recorded number of cases occurred in the period 1943-45 during the interval of active warfare in the European Theater, particularly during the Italian campaign. That period corresponded to the time during which the heaviest fighting occurred and in which the greatest number of casualties were sustained. This was to be expected as the cause of organizing hemothorax is, as previously pointed out, traumatic in practically all instances. Complete statistics concerning the specific wounds of this warfare are not as yet available, but a reasonable estimate of the incidence of cases of penetrating wounds involving the chest which survived to receive definite treatment compared to the total of all treated wounds is in the region of 1 to 2%. Of these, practically all showed a greater or less degree of hemothorax; and of the hemothorax cases between 25 and 40% showed the criteria for establishing a diagnosis of organizing hemothorax. This is in marked contrast to the incidence of the lesion following hemothorax incurred by thoracic injuries in civilian life. The incidence of hemothorax following trauma in civilians is far less. Unfortunately such figures have not as yet been accurately formulated from existing data; but it is the opinion of the author as well as others who have frequently to deal with thoracic trauma that, in civilian practice, organizing hemothorax

occurs in considerably less than 3% of hemothorax cases. The reason for this, as will be noted later, lies in the difference in the ordinary types of wounds encountered in military and civilian activity.

4. Pathogenesis and Pathologic Physiology. It has just been stated that organizing hemothorax is practically always secondary to some well-recognized form of chest trauma which produces hemorrhage into the pleural cavity. Usually the trauma is severe, such as penetrating wounds made by sharp instruments or missiles, and crushing injuries to the chest wall. In practically all instances there is damage of some extent to the contained lung. The essential feature, however produced, is an intrapleural hemorrhage arising either from the vessels of the thoracic wall mediastinum, or from the traumatized pulmonary parenchyma. The extravasated blood in the pleural cavity gravitates to the most dependent portion of the pleura and subsequently undergoes partial or complete clotting, forming a more or less solid jelly-like mass which compresses the adjacent lung tissues against the chest wall or mediastinum. Any degree of compression of the lung may occur. The entire lung may be involved and thus rendered atelectatic and airless, but more usually the process is confined to one lobe, in most instances the lower. The clot thus formed thereafter undergoes a process of invasion by connective tissue fibers from the surrounding pleural structures and ultimately forms a completely organized clot which effectively and permanently impairs activity in the lung which it overlies. The end-result, so far as the pulmonary function is concerned, is a marked reduction in the amount of functioning lung tissue. The vital capacity is reduced in direct proportion to the extent of pulmonary tissue involved which is in turn determined by the extent of the clot. Since the lung in the affected area cannot aërate, the pulmonary reserve, as indicated by the vital capacity, is diminished. The diminution in the pulmonary reserve

may not be immediately apparent under ordinary circumstances, that is, under the normal circumstances of a sedentary existence; but it is noticeable when an increased physiologic demand is put upon the respiration, as in unaccustomed physical exertion. The deficiency becomes noticeable by a more rapid onset of the symptoms associated with anoxia, namely dyspnea, fatigue, and a slower rate of respiratory recovery.

thorax, then, is an intrapleural, extrapulmonary hemorrhagic clot of variable dimensions, which as time progresses becomes organized by the ingrowth of fibrous connective tissue from the adjacent pleural structures (Figs. 1 and 2). The sequence of events is first the extravasation of blood usually in the form of a single massive hemorrhage into the pleural cavity. Sometime thereafter the fluid blood becomes clotted and the process of

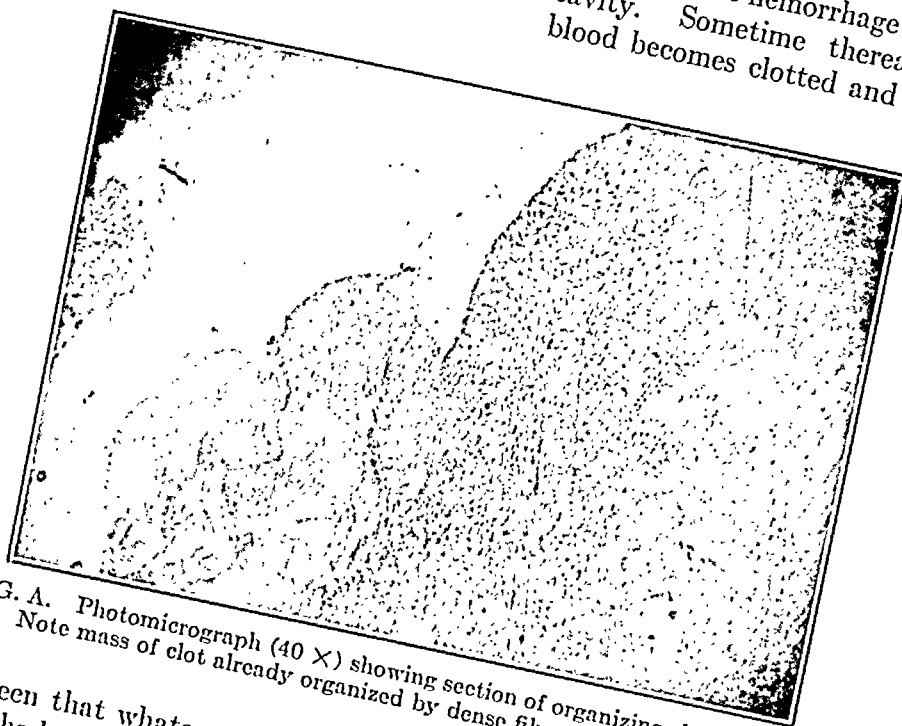


FIG. 1.—Case G. A. Photomicrograph (40 X) showing section of organizing clot removed at operation. Note mass of clot already organized by dense fibrous tissue (right).

It will be seen that whatever anoxia is produced by the lesion is primarily of the anoxic type, and is responsible for the presence of the associated signs and symptoms. Obviously, the severity of the symptoms will be in proportion to the degree of anoxia produced by pulmonary compression from the clot. As in other disabilities affecting all or a portion of the lung, the remaining functional lung tissue compensates to a certain extent for the loss of the affected part. But this compensation can at best be only partial, so that the physiologic limit of the respiratory reserve (measured by the vital capacity) is lowered considerably and permanently, and remains so unless the condition is corrected.

The essential lesion of organizing hemo-

connective tissue ingrowth begins. This ingrowth is not uniform throughout the clot, proceeding faster in some areas than in others, so that unless the clot is very small, multiple loculi may be formed containing shreds and strands of fibrin and clotted blood. Ultimately it is probable that the entire mass becomes fibrosed and undergoes contracture.

The microscopic picture of the essential lesion depends upon the age of the condition. Initially the process shows only the clot with a network of fibrin strands in which the cellular elements are embedded lying between the pleural layers of mesothelium. Later the typical process of inflammation is present with the appearance of polymorphonuclear cells, microphages, lymphocytes, phagocytic dissolu-

tion of the fibrin, and laking of the red blood cells. Still later, as organization progresses, there is proliferation of the fibrous connective tissue cells into the clot mass together with ramification of the endothelium of the blood-vessels. Ultimately the connective tissue cells begin to show contracture of their processes and the greater part of the mass assumes the appearance of solidly organized connective tissue. If, as very frequently happens, the clot contains infectious material (bacteria or foreign bodies), the inflammatory reaction is greatly accentuated and the inflammatory products

ral infections in cases of organizing hemothorax vary somewhat with the conditions under which the wound is inflicted, but in the main are fairly constant. *S. aureus* is the most frequently recovered, and next in order *B. hemolytic streptococcus* and colon bacillus. Mixed infections are relatively frequent, depending, as stated previously, upon the conditions of infliction and the type of the wound.

Precisely what factors determine whether or not clot formation will occur in blood extravasated into the pleural cavity are not known. It is probable that the process is determined to a large extent by

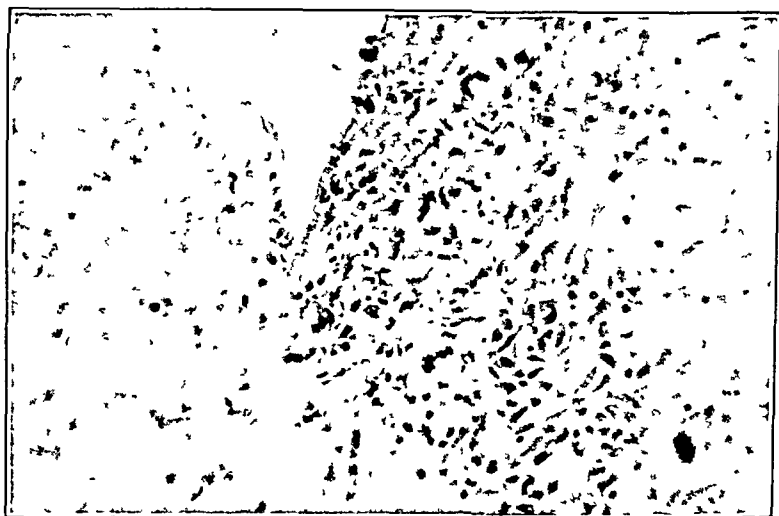


FIG. 2.—Case G A. Photomicrograph (97 X) showing section of organizing clot removed at operation
Note ingrowth of fibrin (left) by connective cells (right).

greatly increased. It is probable that the process of organization of the clot is accelerated by the presence of inflammatory agents and the amount of associated fibrosis is augmented. The amount of inflammatory process present will depend largely upon the character and number of bacterial agents producing it.

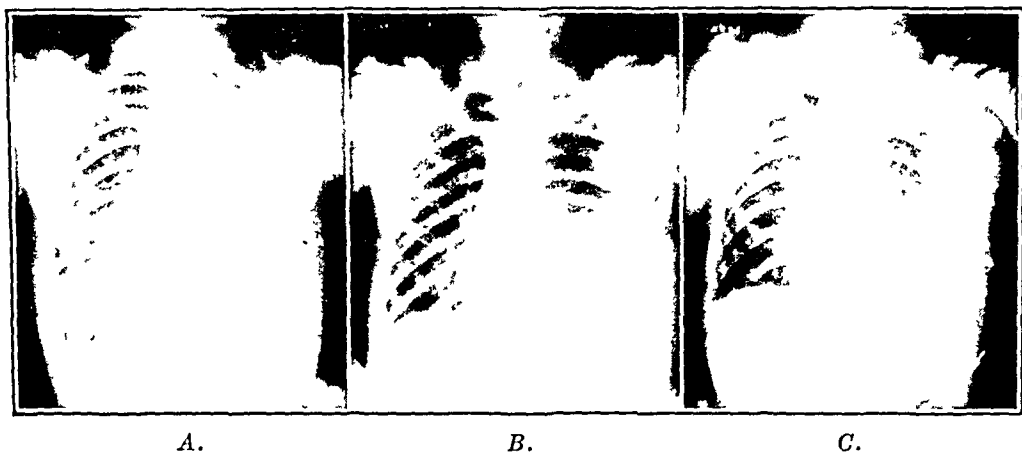
Practically all penetrating wounds of the pleura are contaminated since the agents producing them are rarely sterile or even surgically clean. However, infection resulting from such contamination occurs in considerably less than half the cases. The bacterial agents producing intrapleu-

the presence of contaminating agents and the character of the wound. There is no doubt that intrapleural clotting occurs with far greater frequency in instances of thoracic trauma encountered incident to warfare than in those observed in civilian life. In the former, the wounds are considerably more extensive, involving as a rule larger areas of lung and chest wall, and gross contamination with pathogenic organisms and the presence of foreign bodies are much more frequent. Intrathoracic wounds in civilian life are usually inflicted with relatively cleaner agents of penetration which, as a rule, involve

only small areas of pleura and pulmonary parenchyma. It is probable that extensive injuries of the lung parenchyma increase the probability of intrapleural clotting by the liberation of thromboplastic substance which the lung is known to contain in considerable quantity. The rôle of contaminating agents in the clotting process has been subject to much speculation, and in general the consensus of qualified opinion is that certain bacterial contaminants predispose to premature clotting. Frequently mentioned in this connection is the most common contaminant isolated from cultures of intrapleural clot,

and (2) localizing, resulting from compression of the affected lung.

The constitutional manifestations of organizing hemothorax are indistinguishable from those incident to pleural reaction from liquid blood or infection in the pleura and are in general those incident to any inflammatory reaction. There is usually a moderate elevation of the temperature (99° to 101° F.) with a proportional concomitant rise in the pulse rate and respiration. Leukocytosis of some degree is present and there is more often than not an associated anemia of the hypochromic microcytic variety. These



A.

B.

C.

FIG. 3.—Case G. A. A, Roentgen ray showing postero-anterior view of chest on October 22, 8 days after injury. Aspiration at this time showed blood contained in pleura to be unclotted. B, Roentgen ray showing postero-anterior view of chest on November 8, 23 days after injury. Repeated aspiration showed no recovery of fluid blood indicating that blood clotting had occurred. Note obscuration of entire lower left lobe. C, Roentgen ray showing postero-anterior view of chest on November 17, 1st day postoperative (aspiration November 16, 31 days after injury). Note how decortication process performed at operation has allowed reexpansion of lower left lobe.

S. aureus. It is not unlikely that the clot process is in the usual case materially hastened by both factors. Clotting may occur immediately succeeding the hemorrhage or may be delayed until a considerable period has elapsed. It is presumed that organization of the clot begins as soon as the clot has formed and proceeds until the process is more or less complete.

5. Signs and Symptoms. From the foregoing it will be recognized that the signs and symptoms of organizing hemothorax can be generally classified into 2 groups: (1) constitutional, resulting from body response from reaction within the pleura;

manifestations vary considerably depending upon whether or not there is an infectious process present in the affected pleura coincident with the clot.

The localizing signs and symptoms are of considerably more significance. These arise in the main from pressure by the clot upon the lung and adjacent pleural structures, and vary in severity with the amount of involvement. Usually the chief complaint is that of dyspnea upon moderate effort, pain or a sense of oppression in the affected side, and persistent cough. Examination reveals limitation of respiratory motion over the affected side

with widening of the intercostal spaces when the contained clot is large and in the process of early organization. Tactile fremitus is decreased and the apex beat is shifted away from the affected side. Loss of normal pulmonary resonance and diminished vocal fremitus are present.

6. Diagnosis. The clinical signs are essentially those of fluid in the chest and except for the information available by Roentgen ray and aspiration, are indistinguishable from it. Usually chest films taken in the postero-anterior and lateral views show a diffuse haziness over the whole or part of the affected side of the chest with collapse of the lung of varying degrees. Air is commonly present but not

affected side. The diaphragm on the side of the lesion is in the later stages elevated above its normal position. Fluoroscopy will confirm a restriction of respiratory movement in the chest wall and particularly in the diaphragm. This restriction is due presumably to the pressure and tension produced by the progressive formation of adhesions as the clot begins to organize between the various pleural structures, lung, thoracic wall and diaphragm. Frequently loculi formed by organizing strands of fibrin and containing fluid are noted, and when present are indicative that clotting has occurred. It is of particular importance in cases suspected of developing organizing hemo-



FIG. 4.—Roentgen ray showing postero-anterior and lateral views of chest at the time of discharge from the hospital on November 27 (11 days postoperative, 42 days after wounding). Note lung lobe has remained expanded and has progressively cleared.

invariably. The area of pulmonary collapse is frequently noted in the most dependent portion of the pleural cavity and affects the lower lobe. Early in the course of the lesion there may be, if sufficient fluid and air are present, widening of the intercostal spaces on the side of the lesion with a perceptible shift of the mediastinum to the side opposite. The fluid level, depending upon whether or not air is present, may or may not be sharply defined. As the process of organization proceeds, the fluid level which was formerly quite sharply delineated tends to become diffuse and hazy, the interspace of the thoracic cage narrowed, and the mediastinum shifted back toward the

thorax to have lateral as well as antero-posterior Roentgen ray studies of the chest since exact localization and determination of the extent of the lesion are impossible without them. Furthermore, it should be emphasized that repeated Roentgen ray studies are essential to follow the progress of the lesion as well as any complicating features. These studies should be performed serially and rarely more than a week apart, both before and after surgical information.

Possibly the most important single diagnostic feature in establishing diagnosis of the lesion is findings on aspiration. In general it can be stated that when repeated aspiration of a pleural cavity showing clini-

cal signs and Roentgen ray evidence of a sizable collection of fluid shows negative results, a presumptive diagnosis of organizing hemothorax should be made. In fact, when no fluid is recovered from a site in the pleura where fluid is diagnosed from the extant clinical and Roentgen ray findings, it must be presumed that the fluid blood has become clotted and the condition of organizing hemothorax obtains. Nor is it necessary that no fluid whatever be obtained on repeated aspiration to establish the diagnosis. Very frequently small quantities of fluid blood may be recovered, but the important consideration is that the amount of fluid blood recoverable is minimal in compari-

find small macroscopic clots present in the fluid. This is good presumptive evidence that a more extensive intrapleural clot exists. The appearance of strands of fibrin in the aspirate is also considered of similar significance.

In summary of the foregoing, it may be stated that the diagnosis of organizing hemothorax rests upon demonstration of the following features:

1. The existence of a penetrating intrapleural wound usually extensive in which a concomitant extravasation of blood would be expected.
2. Clinical signs and Roentgen ray evidence indicating the presence of fluid in the pleural cavity.

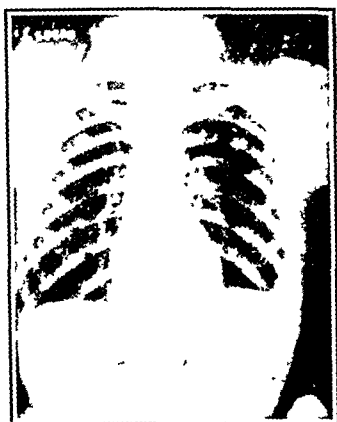


FIG. 5.—Case G. A. Roentgen ray showing postero-anterior and lateral views of chest on December 11 (2 weeks after discharge from hospital, 25 days postoperative, 59 days after wounding). Note that lung is practically normal in appearance.

son to the amount which should be recoverable by correctly performed aspiration in the absence of intrapleural clotting. It is seldom that the clotting process when it occurs in the pleura is entirely complete. Usually there exist small loculi containing laked fluid blood which may be aspirated if the containing loculi are directly penetrated by the needle. The fluid in most instances in laked blood may be seropurulent or frankly purulent in character when infection has occurred. It is obviously important to examine all aspirates bacteriologically for the presence and character of infective organisms. It is not uncommon, when small amounts of blood have been recovered during aspiration, to

3. Failure to recover fluid blood upon repeated aspiration or recovery of only small amounts containing macroscopic clot.

4. Signs and symptoms of lowered vital capacity due to lung compression.

5. The presence of pyogenic organisms in the pleural aspirate.

6. Specific Roentgen ray changes progressively revealed in serial films showing persistent shift of the mediastinal structures, elevation of the diaphragm, and haziness of a previously well-defined fluid level.

7. Considerations on Treatment. The treatment of organizing hemothorax may be classified into 2 general categories,

conservative or radical—the choice depending almost entirely upon the amount of anatomic and functional involvement. With minimal lesions in which only a small amount of lung parenchyma is concerned and the vital capacity of the individual only slightly reduced, a conservative regimen is advisable. Where the amount of involvement is small, considerable improvement takes place, leaving as a rule only a moderate amount of pleural thickening and little ultimate impairment of the function. The most frequent complication in such instances is the occurrence of empyema which should be watched for, and in the event of its incidence, be drained in the usual manner. Results under conservative treatment in properly selected cases are almost uniformly good.

It is in instances in which the lesion has involved a considerable amount of lung parenchyma with proportionate reduction in vital capacity and respiratory function, that radical surgical treatment must be considered, for, if the condition is allowed to continue without intervention, permanent pulmonary disability results proportional to the extent of the lesion. It has been arbitrarily stated by those whose experience in the treatment of organizing hemothorax has been considerable that, when the organizing clot has rendered functionless an estimated 20% or more of the functional lung tissue, radical surgical treatment should be instituted. Most of those observing the results in the treatment of a large number of cases are in general agreement with this concept. Once the decision to employ radical surgical treatment has been made, the time at which operation is to be carried out is a consideration of the greatest importance as will be apparent from the subsequent brief discussion concerning the technical procedure itself.

It is not within the scope of this writing fully to outline detailed surgical technique. There are, however, certain aspects of the operative procedure that bear such an intimate relation to the ultimate re-

sults that they cannot be conscientiously omitted. Pre-operative preparation is particularly important. Not infrequently the patient has coincident injuries which must receive treatment. It is unwise to attempt the requisite surgery until such time as the patient's general status is near the optimum point. Particular attention should be devoted to the elimination of all extant nutritional problems and infectious processes. The hemoglobin level should be approximately normal, and, if deficient, replenished by adequate transfusion. Immediately prior to operation, a quantity of whole blood (2000 cc.) should be available for transfusion during operation and the postoperative period.

8. Technique of Operation. The operative procedure itself consists in opening the pleural cavity, under endotracheal anesthesia, through a thoracotomy incision so placed that it will adequately expose the area involved by the organizing clot. The placement of this incision is previously determined by careful study of antero-posterior and lateral films of the chest. Occasionally it is possible to gain sufficient exposure by incision through the intercostal space but usually the removal of a rib is essential. Upon opening the pleura, particular care must be taken to avoid injury to any underlying lung which may be adherent to the chest wall. When the pleura is penetrated, the pocket, whose walls are formed of organizing clot and whose content is composed in most instances of serosanguineous or sanguinopurulent fluid and bits of free-floating fibrin, is exposed. The content of the pocket is evacuated by suction and sponging, and its limits and the character of its wall examined. The wall is then incised at some point over the underlying lung and by blunt and sharp dissection the visceral pleura of the lung is identified. If this maneuver is properly carried out a plane of cleavage is established between the visceral pleura and the overlying fibrous wall of the organizing clot so that the latter, by careful dissection, can be entirely stripped from the lung which it

compresses. The procedure is progressively continued until the entire area of lung compressed and encompassed by the clot is freed and the restricting wall of fibrous tissue removed. The lung is inflated under positive pressure by the anesthetist to test its expansibility and air continence and the pleural cavity thoroughly lavaged with normal saline to remove residual blood. Drains are inserted in the dependent portion of the pleural cavity and these are immediately connected to water seals. The chest is closed in the usual manner, if the procedure has been properly performed, the lung, which had been previously compressed, reëxpands completely and remains so, and the individual thereby regains practically the entire amount of functional lung tissue.

9. Special Features Concerning the Operative Treatment. Several features concerning the actual surgical procedure are noteworthy. It has already been stated that the time after injury at which operation is carried out is of considerable importance. This is based on the fact that once the clot is formed, it becomes progressively more organized by the ingrowth of fibrous tissue from adjacent pleural structures. If operation is carried out too soon, that is, before there has been sufficient ingrowth of fibrous tissue, the procedure of peeling or decorticating the lung is made technically extremely difficult, due to the fact that the clot is too friable and separates away from the visceral pleura in very small strips. If, on the other hand, the organizing process has been allowed to progress for too long a period, the overlying clot becomes too firmly adherent to the visceral pleura of the lung and separation causes injury to the visceral pleura and underlying lung, and is attended by far more hemorrhage. In either case the establishment of a plane of cleavage between the clot and visceral pleura is extremely difficult and the decortication procedure rendered much more complicated. The time at which decortication should be performed has been a matter of much speculation. It has been

more or less established empirically that the optimum time for operation in the usual case is somewhere between the 3rd and 5th week after clotting has been shown to have occurred. Under ordinary circumstances the decortication performed within this period will result in the establishment of a satisfactory line of cleavage between the clot and the visceral pleura so that the technical process is not too difficult. Great care must be exercised to avoid injury to the vital structures contained in the mediastinum, particularly the great vessels. If the lung parenchyma is inadvertently penetrated as it frequently is during the procedure, the area of parenchymal penetration should be closed at once with the fine silk suture.

Before closing the chest, the surgeon should see that the lung is entirely expanded and fills all of the pleural space. Lavage of residual hemorrhage incident to the peeling process is important and has been mentioned elsewhere. The establishment of adequate drainage is important. This can usually be effectively accomplished by the proper placement of a fenestrated rubber tube of medium caliber in the posterior pleural gutter, and a secondary drainage tube consisting of a catheter placed through the anterior chest wall into the pleura. The fenestrated tube serves to remove accumulations of fluid while the catheter provides a conduit for any remaining air. In each case the tubes should be immediately connected with water seals to allow the lung to become functional as soon as possible. It is the general practice to remove the tubes when there is no longer any fluctuation noted in the water seals.

10. Postoperative Complications. The patient must be carefully watched for postoperative complications. Empyema is a frequent complicating factor which if it appears is treated by drainage in the usual manner. Bronchopleural fistula may occur in which event subsequent operation may be necessary for closure. By far the most frequent untoward postoperative sequel in atelectasis

of the affected side and every effort should be made to forestall its appearance. This can, in most instances, be accomplished by seeing that the drainage tubes are kept clear and by preventing accumulation of secretion in the bronchial tree. In the event that postoperative atelectasis does supervene, bronchoscopy and aspiration usually are necessary. In the majority of instances, however, the procedure of decortication in carefully selected cases of organizing hemothorax should yield uniformly good results.

11. Case Presentation. The following case of organizing hemothorax is presented as showing the typical features with regard to diagnosis, radical surgical treatment and ultimate result. The accompanying illustrations are taken from this case.

G. A., a 19 year old negro male, was admitted Oct. 22, 1945, to the University Surgical Service of the Detroit Receiving Hospital with a stab wound of the left chest. Examination on admission showed that the wounding instrument, a knife, had penetrated the chest wall in the left scapular line at the level of the fourth interspace. Chest findings confirmed by Roentgen ray revealed the presence of a left hemothorax. Patient was put on observation and conservative treatment. Later, on the day of admission, he complained of dyspnea and the left chest was aspirated of 650 cc. of blood with improvement of symptoms. On October 29, his chest was reaspirated and 850 cc. of laked blood recovered. On November 3, his temperature, pulse and respirations were elevated and reaspiration of the chest recovered 350 cc. of bloody fluid. Cultures returned

upon previously aspirated fluid showed the presence of a non-hemolytic streptococcus and *S. albus*. The patient continued to run a fever in spite of the administration of penicillin in large doses. Roentgen ray studies showed the presence of fluid but none was recovered on 3 successive aspirations. A tentative diagnosis of organizing hemothorax involving the left lower lobe was made and the patient prepared for surgery. The operation was performed on November 16, 25 days after being wounded.

The pleural cavity was approached through a standard thoracotomy incision by removal of the sixth rib. The entire left lower lobe was found to be tightly compressed against the mediastinum by an extensive organizing clot whose walls were composed of ingrowing connective tissue and whose central portion contained laked blood and many light adhesions. The compressed lower lobe of the left lung was completely decorticated, leaving a practically normal visceral pleura beneath. Subsequently the lung was expanded under positive pressure and filled the entire pleural cavity. Drains were inserted and the chest closed. The entire procedure lasted 2 hours and 45 minutes. The patient received a 500 cc. transfusion of whole blood during operation and left the operating room in good condition.

The postoperative course was uneventful. The lung remained well expanded as shown by interval Roentgen rays. Drains were removed on the 4th postoperative day. The patient shortly thereafter became ambulatory and practically asymptomatic. He was discharged to the out-patient department on December 28. When last seen in the out-patient department, on Feb. 22, 1946, he was asymptomatic and had resumed his full normal occupational activity.

REFERENCE

TUTTLE, W. M., LANGSTON, H. T., and CROWLEY, R. T.: The Treatment of Intrathoracic Wounds. Surg., Gynec. and Obst., 81, 158, 1945.

BOOK REVIEWS AND NOTICES

THE AUTONOMIC NERVOUS SYSTEM. By ALBERT KUNTZ, PH.D., M.D. Third ed. Pp. 687; 91 illus. Philadelphia: Lea & Febiger, 1945. Price, \$8.50.

Of the relatively few books on the autonomic nervous system, this has long been regarded as the best. Research along this line has been progressing so rapidly that already many views are changing. Nevertheless, Dr. Kuntz has nicely reviewed the literature up to 1942 and has integrated the mass into a readable story.

The general outline includes, first, general anatomic and physiologic considerations, second, a detailed review of the systems supplied by the autonomies and, third, the rôle of these nerves in the pathogenesis of various diseases. Lastly he reviews the surgical approaches and attempts an evaluation of their efficiency.

The 100-page bibliography indicates the labor spent in preparing this volume. Because of this list it is obvious that the book is a good reference text. J. C.

THE EXTREMITIES. By DANIEL P. QUIRING, PH.D., Head of the Anatomy Division, Cleveland Clinic Foundation and Associate Professor of Biology, Western Reserve Univ.; BEATRICE BOYLE, Artist, Cleveland Clinic Foundation; ERNA L. BROUSH, M. A., Anatomy Division, Cleveland Clinic Foundation; and BERNARDINE LUFKIN, A.B., former Secretary, Research Division, Cleveland Clinic Foundation. Pp. 117; 107 illus. Philadelphia: Lea & Febiger, 1945. Price, \$2.75.

In order to simplify the relations of the muscles, nerves and arteries of the upper and lower extremities, the authors have depicted 101 muscles, each in a separate diagram. In each diagram, besides the origin and insertion of the muscle the chief arterial and nervous supplies are shown. On 52 of the muscles the motor points of the muscles as found in normal subjects are presented. The plates of the separate muscles will be welcomed by medical students and

will serve as a quick source of reference to all concerned with function and dysfunction of the limbs. The position of the motor points should be especially useful to physical therapists. W. A.

THE CHEMISTRY OF ANESTHESIA. By JOHN ADRIANI, M.D. Pp. 502; 45 illus. Springfield: Thomas, 1946. Price, \$7.00.

ANESTHESIOLOGY is coming of age as a medical specialty. This seasoning process has been accomplished in part by books concerned with various fundamental aspects of the field. There are volumes devoted to the physiology of anesthesia, and to the pharmacology of anesthesia. Here now is the chemical approach to problems of anesthesia.

Adriani has done a splendid job on the whole. There are lucid presentations of such important topics as carbon dioxide absorption, the application of physical laws of gases to clinical anesthesia, explosion hazards, basic chemistry of such substances as ethers, aldehydes, esters, etc. The book is divided into 3 large sections: inorganic chemistry, organic chemistry and biochemistry. In the latter 2 sections there is a good bit of pharmacology; but, as the author points out, this is inevitable.

There is nothing new in the volume. It is merely a compilation of information designed for the information of clinical anesthetists. As such it has an appeal limited primarily to this group. For them, however, it can be unhesitatingly recommended. R. D.

PATHOLOGY IN SURGERY. By NATHAN CHANDLER FOOT, A.B., M.D., Professor of Surgical Pathology, Cornell Univ. Medical Coll.; Surgical Pathologist, New York Hosp. Pp. 512; 368 illus. Philadelphia: Lippincott, 1945. Price, \$10.00.

"This book is intended as a guide to the surgical pathology of those disorders in which operations are carried out and organs or other specimens removed . . ." It

can serve as a text for a student course in surgical pathology or as a handbook for those training in pathology. There are few good textbooks in this special field and a new one is a welcome addition.

The book begins with a discussion of the methods used in surgical pathology, followed by chapters on the general pathology of inflammation, wound healing, and tumors. The largest part of the book is devoted to the special pathology of various tissues and organs: fibrous tissue, cartilage and bone, bone marrow, lymph nodes and spleen, alimentary tract, breast, nervous system, and skin—to list a few examples. Important lesions are discussed in considerable detail, others are merely mentioned. As the author states, the book is not intended to “be too comprehensive in the theoretical consideration of all the aspects of the lesions that are described.” Adequate bibliographies are given for those desiring to pursue the details of any special subject.

Practically all of the illustrations are original. They are of very uneven quality. A few are excellent (notably those from the Army Medical Museum), most are fair to poor. The color reproductions are unbelievably bad. Most are unrecognizable even with the legends and the book would be greatly improved by the omission of many. One serious error is the inclusion of some photographs of breast tissue twice, once to illustrate intractable papillomas, once to illustrate malignant change.

The book is published in the new double-column style for easy reading and is bound as a companion to Karsner's “Human Pathology.” It will surely prove useful to students and pathologists alike.

W. S.

COLLOID CHEMISTRY, THEORETICAL AND APPLIED. Collected and edited by JEROME ALEXANDER. Vol. VI. General Principles and Specific Industries, Synthetic Polymers and Plastics. Pp. 1215; fully illus. New York: Reinhold, 1946. Price, \$20.00.

THIS 6th volume of Dr. Alexander's encyclopedia of colloid chemistry contains 71 contributed papers by experts in their chosen fields. The subjects are approximately evenly distributed between applications of colloid chemistry in industrial and techno-

logic processes and the enlarging fields of resins and plastics.

Although even plastics have some biologic interest (with reference to proteins), among the chapters of more direct interest to medicine and biology are excellent discussions of the “Mass Spectromotor and Its Applications” (containing many interesting applications besides those of isotopes in the study of intermediary metabolism) and “Some Practical Aspects of Electron Microscopy.” The latter article by Drs. V. K. Zworykin and J. Hillier is exhaustive and well illustrated. There is also a comprehensive review by Dr. F. C. Combes on “The Skin and Its Technical Hazards,” which should be of particular interest to dermatologists. Other subjects which may prove useful to biologists are “Adsorption from Solution by Activated Carbon” and “Removal and Recovery of Proteins by Water-soluble Lignin.”

As a timely inclusion there is a final chapter on nuclear energy and atomic fission. This is a very readable account, contributed by the editor.

Alexander's “Colloid Chemistry” from its inception in 1926, when Volume I was published, has been recognized as a standard work. The individual articles are valuable and authoritative. However, the time has been reached when it would seem desirable to publish an index volume, since it has become practically impossible to “readily place one's hand” upon a particular subject in this large reference work. While no doubt little can be done about it, *opera* such as these appear destined only for the shelves of relatively large libraries—\$20 a volume for a periodically published work is not an easy cost for the average interested reader.

D. D.

BIOENERGETICS AND GROWTH. With Special Reference to the Efficiency Complex in Domestic Animals. By SAMUEL BRODY, Ph.D., Chairman, Committee on Growth and Energy Metabolism College of Agriculture, Univ. of Missouri, Columbia, Mo. Pp. 1023. New York: Reinhold, 1945. Price, \$8.50.

THE present work by Dr. Samuel Brody, an active member of the research staff of an agricultural and animal husbandry experiment station, is concerned with the inter-

related subjects of growth, efficient utilization of energy and the processes of aging in farm animals. The fields covered by the book are dominant trends in recent biologic investigation, making the treatise very timely.

The farm animals, fowl, hog, cow and horse, have been neglected owing to their size as laboratory subjects, but they should be of particular interest as producers of foodstuffs—egg, milk, meat—as producers of muscular work (the horse), and, finally, as sources (the bovines) of hormones for replacement therapy in man. Dr. Brody's volume is an integrated compilation of data secured over many years in a coöperative effort by the staff of the Missouri Agricultural Experiment Station (Univ. of Missouri), made possible by endowment by the Herman Frasch Foundation for Research in Agricultural Chemistry. The writing of the volume was aided by a fellowship grant of the John Simon Guggenheim Memorial Foundation.

The underlying theme is the farm animals as energy consuming and producing machines. Such subjects as animal calorimetry, surface area (of cattle), growth, production of milk, etc., and production efficiency correlated with aging are discussed in detail. Numerous tables and figures make for a well documented, encyclopedic text, not too easily read.

The whole work bears the stamp of authority, though somewhat biased by personal viewpoint. Dr. Brody's scholarly volume appears destined to serve as a source of information in this important field of "bioenergetics"—growth and production efficiency in farm animals, and is worthy of high commendation. D. D.

and the author has arranged a brief discussion of the various lesions of the tract. As the author states in the Preface, foreign bodies in the gastro-intestinal tract have been emphasized because of his unique experience in that field. It is not a comprehensive treatise. As a small monograph on certain phases of diagnosis or treatment it follows the general trend of many of the more recent medical publications. With the text the author has collected a group of excellent illustrations.

The publisher is to be congratulated no the excellence of the physical qualities of the book. Its composition, the excellent paper, the print and the illustrations maintain the high standard that Mr. Thomas has set for his books. E. P.

TRATADO DE CARDIOANGIOLOGIA. By PEDRO A. P. TAPELLA. Pp. 946. Lopez & Etchegoyen, SRL, 1946.

THIS well-documented volume presents in considerable detail the subject of cardiovascular diseases in 3 large divisions. More than a third of the book is given to generalities of cardiovascular anatomy, physiology and semeiology; a full half of the space is occupied by Part 2, The Cardiopathies; the 94 pages of Part 3 are given to Treatment. The subdivisions are much as one would find in a similar work in English. The 2066 references in the bibliography are to American articles in such a large proportion that those nationalistically minded will be much pleased. They should not assume, however, that this is necessarily a true index of the importance of American contributions to the subject and not merely indicating, perhaps, the current urge for writing and the opportunity for publication in this country. E. K.

ROENTGEN DIAGNOSIS OF DISEASES OF THE GASTROINTESTINAL TRACT. By JOHN T. FARRELL, M.D., Clinical Professor of Radiology, Graduate School of Medicine, Univ. of Penna.; Radiologist, Lankenau Hosp. Pp. 271; 190 illus. Springfield, Ill.: Thomas, 1946. Price, \$5.50.

THIS book is a manual which will serve as a guide to some of the procedures carried out in the examinations of the various portions of the gastro-intestinal tract. The standard nomenclature of diseases is utilized

WESTERN RESERVE UNIVERSITY CENTENNIAL HISTORY OF THE SCHOOL OF MEDICINE. By FREDERICK CLAYTON WAITE. Pp. 588; 16 illus. Cleveland, O.: Western Reserve Univ. Press, 1946. Price, \$6.00.

DR. WAITE's book is the first history to be written of this important School of Medicine. It will necessarily be of especial interest to its friends and graduates and also is a valuable local record. He has gone

further, however, than portraying the institution with which he has been connected for more than half of its existence. The first 4 chapters give a brief survey of early medical instruction in this institution; other chapters consider rival institutions, and 9 appendices that of diverse matters. In fact, as the events portrayed are also considered in their causal and relative relations, the book may be regarded as covering much of the history of medical education in Ohio.

E. K.

CLINICAL ELECTROCARDIOGRAPHY. By DAVID SCHERF, M.D., F.A.C.P., Associate Professor of Medicine; and LINN J. BOYD, M.D., F.A.C.P., Professor of Medicine, New York Medical Coll., Flower and Fifth Ave. Hosps. 2nd ed. Pp. 267; 243 illus. Philadelphia: Lippincott, 1946. Price, \$8.00.

THE authors state that in preparing this edition they have been primarily interested in a close correlation between electrocardiographic findings and their clinical evaluation and that for this reason emphasis has been directed to the practical applications of electrocardiography, to the clinical aspects of disturbances of rhythm and to therapy.

The discussion of abnormalities of cardiac mechanism is excellent and may be read with profit by the beginner and with interest by those who have had wide experience in this field. The only adverse comment this Reviewer can offer concerning this part of the book is that a few of the tracings made with the machines of the past generation do not reproduce well enough to be informative.

With regard to those sections which deal with the most important aspects of clinical electrocardiography, such as the changes produced by intraventricular conduction defects and various forms of myocardial damage incident to coronary disease, the book leaves much to be desired. The authors have apparently adhered to the one precordial lead school of thought and very few of the illustrations include more than one lead of this type. The inadequacy of the 4 lead electrocardiogram for the display of bundle-branch block and lesser grades of intraventricular conduction defects as well as localized areas of damage involving certain parts of the ventricular wall presenting toward the anterior surface of the chest has

apparently not been recognized. Likewise the importance of the patterns of change that may be recorded when leads are made with the exploring electrode placed on an adequate number of precordial positions has not been stressed. Thus, in certain important respects, the book is not abreast of current thought and practice in electrocardiography.

C. W.

THE SULPHONAMIDES IN THEORY AND PRACTICE. By J. STEWART LAWRENCE, M.D. (EDIN.), M.R.C.P., Physician, Haymeads Emergency Hosp., Bishop's Stortford. Pp. 105; Preface and Bibliography. London: H. K. Lewis, 1946. Price, 9/.

A BRIEF summary of the history, mode of action, pharmacology, clinical use, toxicity, and present status of these drugs, including a bibliography of 323 titles and directions for estimating the sulfonamide content of blood or urine. It is written mainly for the guidance of clinicians and therefore presents for each drug such items as structure, absorption, excretion and distribution in the body, which are not usually available in as concise form as this. American observers may not be ready to accept the author's conclusion that sulfamethazine is the sulfonamide of choice for routine use but the descriptions of clinical use, toxic symptoms, and choice between these drugs and penicillin are worthy of highest commendation.

C. S.

NEW DRUGS. By ARTHUR D. HERRICK. With a Foreword by Dr. Austin L. SMITH. Pp. 295. New York: Revere, 1946. Price, \$4.00.

THIS book is intended to describe and explain the requirements of the Federal Food, Drug and Cosmetic Act of 1938 with regard to the testing of new drugs before they are marketed to the public. The author, evidently a lawyer with considerable experience in this field, sketches the background and scope of the present law interestingly and informingly in 15 chapters (150 pages), and adds an Appendix (116 pages) containing the documents relative to the notorious Elixir Sulfanilamide-Merck-gill, the text of the present Federal law, pertinent excerpts from present state and city laws, and the official rules of the Council on Pharmacy and Chemistry of the

American Medical Association, whose secretary contributed the Foreword. The book should be extremely valuable to those concerned with the making and testing of new drugs. The average physician may also read with profit this account of some of the past shortcomings of his own profession with respect to the use on unsuspecting patients of insufficiently tested new drugs.

C. S.

HOWELL'S TEXTBOOK OF PHYSIOLOGY. Prepared by JOHN F. FULTON. 15th ed. Pp. 1304. Philadelphia: Saunders, 1946. Price, \$8.00.

A NEW edition of this classic is indeed welcome after the lapse of 6 years. The present edition, the first since Dr. Howell's death, has been prepared by Dr. John Fulton with the help of 10 collaborating editors. It shows, as one might expect, an extensive revision with many new and rewritten chapters. Though this has required 187 more pages, the book is not inconveniently larger or heavier. Six of the opening pages are given to a preliminary statement on Historical Background of American Physiology; one might wish that this was several times as long and not limited to "American," especially as it is contributed by the editor. The strength of the Yale faculty in physiologic activities may be inferred from the fact that 15 of the 24 contributors are members of that faculty and all but 3 of professional grade. Though the Preface gives some idea of the main changes, one must study the book itself to appreciate them.

E. K.

ESSENTIALS OF ALLERGY. By LEO H. CRIEP, M.D., Assistant Professor of Medicine and Lecturer in Immunology, School of Medicine, Univ. of Pittsburgh, etc. With a Foreword by ROBERT A. COOKE, M.D., Chairman, Committee on Education, American Academy of Allergy. Pp. 381; 42 illus., 1 color plate. Philadelphia: Lippincott, 1945. Price, \$5.00.

THIS handy little volume fully lives up to its title. Allergy is a rapidly growing field touching every phase of medical practice, but including much that is as yet controversial. A full statement of the subject is far too involved for any except those working in the field. On the other hand, current

textbooks in medicine, surgery and the specialties are inadequate in their presentation of allergy. There has heretofore been a need for a concise and practical text for the use of students and physicians: a need which this volume fully meets. The author has shown excellent judgment in the type and amount of material included and a commendable clarity and order of presentation. There are chapters on the basic principles of anaphylaxis and allergy: the diagnosis and treatment of allergy; hay fever, asthma, nasal and cutaneous allergy; allergy to sera, drugs, bacteria, fungi, physical agents; miscellaneous manifestations of allergy; allergy in children; the diagnostic skin tests for various diseases. There are many illustrative case reports. As Dr. Cooke says in his Foreword, the book "should be a joy to the undergraduate students and the general practitioners for whom it is intended."

R. K.

ASCLEPIUS. A Collection and Interpretation of the Testimonies. Book I. Testimonies and Book II. Interpretations. By EMMA J. and LUDWIG EDELSTEIN. Pp. 479 (I) and Pp. 1304 (II); no illus. Publications of the Institute of History of Medicine, 2nd Series, and Documents, Vol. II. Baltimore: Johns Hopkins Press, 1946. Price, \$7.50 for Book I, and \$8.00 for Book II.

THIS painstaking, scholarly work of the 2 Edelsteins is one of the most monumental that has thus far emerged from the Johns Hopkins Institute. It aims at understanding the problem of Asclepius, both in his aspect of the divine healer and his later rôle, in the final stage of paganism, as "the foremost antagonist of Christ" in the early days of Christianity. The first and larger volume is a Collection of the Testimonies, i. e., Greek and Latin references (fortunately for most of us, with English translations included). They are arranged in 7 categories: (1) Legend (his birth, accomplishments, life character and death, his tomb, and his star, Ophiucus); (2) Descendants; Deification; Medicine; Cult; Images; Sanctuaries. An 18 page *Index Locorum* offers ready reference to the sources. The second volume, the Interpretation of the Testimonies, "aims at an insight into the essence of the Asclepius figure and of the cult attached to him," and also provides a commentary on the "testimony" of the

ancients. The authors stick closely to their subject; they have aimed to avoid such tempting considerations as the nature of the ancient deities, or Asclepius' place among them, comparison of Greek and Roman views with those of other nations, theories of the origin of religion and similar related topics. Asclepius, himself, however, is considered in his human rôle up to classical times and as a god thereafter, also his cult, his images, his temples. Much that has seemed incongruous to the average reader of Greek medical history finds explanation in this entertaining volume and the wealth of scholarly observation and interpretation will make it of permanent value to other scholars. We congratulate heartily the Johns Hopkins Institute on the appearance of this noble product. E. K.

HIPPOCRATIC WISDOM. By WILLIAM F. PETERSEN, M.D. Pp. 263; 77 illus. Springfield, Ill.: Thomas, 1946. Price, \$5.00.

THE laudable purpose of Dr. Petersen's book is to bridge the chasm between the technical dexterity that our recently acquired wars of objective information permit and the integration of the sum of medical knowledge that the patient requires. This, he thinks, can best be done by returning to Hippocrates. His well-known concern with the effect of environmental influences on man naturally led him to Avis Waters Places,

which with some justification he regards as the high point of Hippocrates' objective study of disease. Whether he has read into the text more than the author had in mind, and whether it is correct in appraisal of disease that "the weather comes first," that is indeed another matter. Nor will all accept that Hippocrates was the first meteorologist, the first anthropologist, ecologist and biologic scientist.

By always presenting Hippocrates' own words in roman type, the bulk of the book is in italics. This method brings out various pomp which none but the close student of Hippocrates is apt to realize. For instance, though the Greeks are not regarded as having performed autopsies, Hippocrates is shown to assert that from the dead we know the living and to have recognized a relation between tubercles in the lungs and "curvature and contraction" of the spine. We recognized that the seat of disease had importance, that swimming creatures had to inhale the air in the water, that "male seed passes into the uterus and there commingles with the female seed," and so on through many more examples. Anoxia, reproduction, the medical and surgical clinics, and even theory receive illuminating treatment, which the Reviewer confesses is much more impressive than he had anticipated. This short book has a far greater value than that of entertainment for the historically minded reader. It can advantageously be studied by those medical neophytes who want a perspective for their detailed studies. E. K.

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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

SEPTEMBER, 1946

ORIGINAL ARTICLES

FOLIC ACID (SYNTHETIC L. CASEI FACTOR), AN ESSENTIAL PANHEMATOPOIETIC STIMULUS

EXPERIMENTAL AND CLINICAL STUDIES*

BY CHARLES A. DOAN, M.D.

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THE current era of chemotherapeutic and antibiotic therapeutic triumphs in the field of the infectious diseases has tended to subordinate and overshadow the rôle of optimum nutrition and the importance of an effective mobilization of cellular and humoral immune forces in the resistance economy of the human body. In a world of increasing scientific complexity, with its multiplicity of geographic, social, economic and dietary maladjustments, and with an imminent inevitable upsurge of intercontinental, interracial admixtures of inherited and acquired traits and diseases, it is mandatory to reëvaluate *all* of the factors necessary for maintaining strong bodies and sane mentalities. No longer optional, or to be casually accepted or hoped for, these attributes have become vital to human understanding and happiness, essential to human survival.

It is well within the memory of most of us when really serious and scientific attempts were first made to understand and control the minutia of detail about essential nutritional requirements for optimum growth, development and function—and that progress has been made in the appli-

cation of this knowledge to the solution of specific human disease problems. Biologic and physical chemistry, and the experimental method, are making increasingly possible both direct and indirect controls over the phenomena of health and disease to an extent previously unimagined and unpredictable.

The interplay of bacterial and viral agents among a population comprising individuals with idiosyncratic and variable physical reactions and responses, dependent upon both environmental and inherited or acquired organic characteristics, has proven all too frequently to be disastrous to persons, to nations and to races. Gradually but surely, at least the physical basis for disease prevention and cure is being laid, and the emphasis, therefore, is steadily shifting toward the psychologic, the emotional aspects of human character—for better or for worse, as was emphasized in last year's Jarecki Lecture. It is the more recently acquired knowledge relating to specific molecular nutrition and to the closely related mammalian cellular mechanism of resistance which has been

* The 12th Edwin A. Jarecki Memorial Lecture, Philadelphia, May 17, 1946.

chosen for presentation in this Memorial Lecture.

It is no mere coincidence that our own first studies of living blood and bone marrow cells were initiated in Baltimore some 25 years ago from the standpoint of nutritional control factors. Prior to Dr. Florence R. Sabin's pioneer studies of embryologic blood formation in the living chick blastoderm,³⁷ practically all of the recorded observations had been on fixed and stained preparations of sectioned material, and the only studies on simplified hypocellular adult marrow had been from tissues subjected to severely toxic, destructive influences.⁴⁴ In an adjacent laboratory, McCollum was in the midst of his synthetic dietary studies in rats with special reference to vitamin factors, and we were permitted and invited to observe the influence of various dietary combinations on the hematopoietic equilibrium. But the material was too complex for satisfactory analysis at that time, since no one of the diets studied provided an appreciable marrow hypoplasia in the rat, and the fundamental underlying cellular structure of this organ, under normal adult conditions, was not yet revealed. It became evident early in our work that an easily induced, reversible, non-toxic, hypoplasia of adult marrow must be available for the initial basic study of bone marrow structure and function.¹⁷ An adult avian form was chosen, the pigeon, in order to correlate more directly Sabin's analysis of erythropoiesis in the chick blastoderm. Simple withholding of all solid food for a relatively brief period was found to produce a progressive pan-marrow hypoplasia for all normal elements in the adult pigeon, so that with the resumption of a normal grain diet the prompt but progressive return to normal blood cell formation permitted the observer to note the origins and relationships of the various cell types.¹⁸ Studies followed, in the mammal, as well as the avian species, in the attempt to discover the dietary factors essential for each blood cell type. While Whipple and his asso-

ciates were establishing the essential dietary constituents for hemoglobinogenesis in anemic dogs, the rôle of the nucleotides in granulocytopenia in pigeons and rabbits,¹⁹ and subsequently in human granulopenic states,²⁰ was being explored.

During the *interim* between World War I and World War II, epochal progress was achieved through the combined genius of Whipple, Minot and Castle, not alone through the specific application of their discoveries to the successful treatment of pernicious anemia but, even more, through the establishment of the principle of nutritional deficiency, on either an intrinsic or extrinsic basis, as solely responsible for the cellular maturation arrest, which tissue pathologists had previously interpreted as malignant megaloblastic anaplasia. This revelation has provided a pattern for the approach to many other unsolved medical problems.

Despite the unavoidable diverting of investigational activities to direct war objectives in the immediate past, nevertheless efforts have been gradually intensified, utilizing modern chemical, physical and pharmacologic techniques, to fractionate, isolate, identify and synthesize specific molecules with specific biologic activity, from vitamin complexes and from many other sources. Slowly but surely biologic investigators are separating the active from the inactive, the specific from the non-specific, the innocuous from the dangerous, the stimulatory from the depressant or inhibitory chemical combines which affect living protoplasm. It is down this avenue of diversified investigations, from many individuals and groups with multiple interests and varied objectives, that has come the information, which is focusing attention sharply at the present time on the so-called "folic acid" or "*L. casci* factor" concentrated in terms of human bone marrow activity.

It was inevitable that some confusion should arise temporarily concerning this newest subdivision of the rapidly expanding vitamin B family, due to its wide distribution in nature, its multiple, specific

logic activities, and its variously acquired nomenclature, derived from widely separated groups of investigators. There are 5 designations, which have been commonly and somewhat indiscriminately applied to this group of compounds: vitamin M, vitamin B_c, eluate factor, folic acid and *L. casei* factor. Briefly, their historical derivations may be indicated. In 1935, Day, Langston and associates published the first of a series of communications,^{13,14,15,16,27} describing the development of a nutritional cytopenia in *monkeys* on a vitamin B deficient diet, which could be prevented or corrected by *either* liver extract or brewer's yeast. The essential but unidentified component or components common to these 2 foods was designated in 1938 as "vitamin M."²⁷ Similar studies were started in our laboratory in 1940 in association with Drs. Henry E. Wilson, Jr., Oram Woolpert and Samuel Saslaw, in an attempt to identify the essential hematopoietic factor—more specifically a *granulopoietic* factor—among the growing group of subfractions purified and isolated from the vitamin B complex. Niacin amide, thiamin, riboflavin, calcium pantothenate, pyridoxin, inositol, para-aminobenzoic acid, choline, pimelic acid and glutamine were used as they became available, and individually and collectively they failed to prevent the gradual development of a pan-marrow hypoplasia in the *Macacus mulatta*, when placed on a restricted diet (Figs. 1 and 2). We concluded^{39,60} that the crystalline folic acid obtained from liver by Stokstad⁵² and a highly purified concentrate from yeast^{24,35} each "closely simulated the effect of parenteral liver extract, re-establishing also a normal white cell equilibrium" in our monkeys (Fig. 1). The identity of vitamin M with these folic acid concentrates was suggested and implied.^{39,61} Waisman and Elvehjem⁵⁶ confirmed the antileukopenic effect of a "folic acid"-rich norite eluate concentrate for monkeys in 1943, and Day *et al.*¹² reported in 1945 the successful treatment of "vitamin M deficiency" in the monkey

with highly purified *L. casei* factor, thus establishing the biologic identity of these 2 factors.

In 1939, Hogan and Parrott²² described an anemia in chicks due to an undetermined dietary deficiency, which they designated "vitamin B_c," the "c" being appended to identify the chick species. Its isolation from *liver* in crystalline form was accomplished in 1943 by Pfiffner and co-workers,³³ who stated that "it appears probable that the chick anti-anemia factor B_c, Peterson's eluate factor, and William's 'folic acid' are the same." In 1945 these same investigators³⁴ isolated a second crystalline compound with anti-anemic activity for the chick, this time from *yeast*. The molecular weight, however, was found to be 2.8 times that of vitamin B_c from liver, and it was termed "vitamin B_c conjugate." Typical vitamin B_c was obtained from the "conjugate" on enzyme digestion. Scott *et al.*⁴³ reported that following severe experimental hemorrhage in hens, the intramuscular injection of *L. casei* factor synthetic or of beta-pyracin hastens the regeneration of hemoglobin, and when both are given together the recovery is still more rapid and a higher total hemoglobin level is attained.

Snell and Peterson⁴⁶ in 1940 found that among the requirements for the cultivation of *Lactobacillus casei* was a factor occurring in *yeast*, *liver* and *other natural materials*, which could be adsorbed on norite and eluted therefrom with ammoniacal alcohol. Stokstad,⁵³ and Hutchings, Bohonos and Peterson²⁴ then carried the concentration of this "eluate factor" further, and the latter showed that this concentrate had growth²⁵ as well as anti-anemic activity²⁸ for the chick. Similarities in chemical properties between this "norite eluate factor," factor U of Stokstad and Manning,⁵² the alcohol precipitate factor of Schumacher *et al.*,⁴² and vitamin B_c²² were pointed out. Then Hutchings, Stokstad, Bohonos and Slobockin²⁶ in 1944, using absorption spectra data for identification, grouped all of

these concentrates under the general term "*L. casei* factor."

Mitchell, Snell and Williams²⁹ reported in 1941 that they had obtained from spinach in relatively pure form an acid nutilite, which they called "folic acid," and which they found to be essential for the growth of *L. casei* and *Streptococcus lactis*. Many investigators, thereupon, adopted this euphonious designation as a generic term to cover all substances with similar physiologic properties from whatever source derived. In 1944, however, the originators of this cognomen,³⁰ defined "folic acid" specifically as an essential growth factor for *S. lactis*, thus limiting considerably its strictly literal application to some of the other closely related, but not identical substances.

Daft and Sebrell⁷ in their recent review of this subject (1945) adopted and defined the term "*L. casei* factor" as "any substance having activity for the rat and chick, and either active for *L. casei* or *S. lactis*, or capable of being changed to an active form by treatment with acid, alkali, or any enzyme."

Last August, Angier and his associates¹ announced the synthesis of a compound, without revealing its exact structure, which has ultraviolet and infrared absorption spectra, crystalline structure, and biologic activity identical with the *L. casei* factor isolated in pure form from liver by Stokstad.⁵² Supplies of the crystalline biologic have been necessarily extremely limited in the past, only $\frac{1}{10}$ ounce of active material resulting from a ton of original fresh liver. The relatively more abundant quantities of folic acid of standardized uniform potency now available has stimulated great interest and investigative activity.

Most of the studies have stressed only the anti-anemia and growth stimulating properties of these concentrates, though the work in monkeys recognized from the beginning leukopenia as well as anemia in the vitamin B deficiency state. A few investigators have noted the possible significance of *L. casei* factor for granulo-

cytopoiesis and thrombocytopoiesis, which would seem an even more important clinical attribute of these molecular complexes, if true.

In a series of studies with rats it was found by Welch *et al.*,⁵³ Daft and Sebrell *et al.*,⁴⁷ and Elvehjem *et al.*^{5,56} that agranulocytosis may occasionally occur in the presence of a dietary deficiency only, but the incidence is greatly increased if sulfa drugs, or thiourea and thyroxin⁸ are included in the purified diet. Furthermore, crystalline "folic acid"⁹ successfully prevents or corrects this development. More recently Daft, Sebrell and associates¹² (1945) have reported severe anemia and or granulocytopenia, with bone marrow hypoplasia and congestion in a high percentage of rats fed a purified diet low in pantothenic acid, in contrast to their controls. Only 20% of the rats escaped some form or degree of blood dyscrasia. A few ran a very rapid, fulminant fatal course. The therapy consisted of 200 to 5000 mg. daily of pantothenic acid alone or combined with 25 to 100 mg. of *L. casei* factor. They conclude: "the granulocytopenia, when unaccompanied by anemia, appeared in most cases to be the result of *L. casei* factor deficiency, which for reasons unknown, developed more easily in the absence of pantothenic acid. Folic acid alone did not affect the anemia, and pantothenic acid alone did not affect the granulocytopenia. The anemia responded reasonably well to the combined treatment of pantothenic acid and folic acid. It would seem, therefore, at least a possibility that this anemia was due to the simultaneous deficiency of these 2 factors." Along these same lines, Wright and Welch⁵³ found that feeding rats a highly purified diet containing sulfasuxidine, resulted in a marked reduction in the *liver* stores of folic acid, biotin and pantothenic acid, the other B group vitamins being unaffected. This occurred despite normally adequate amounts of pantothenic acid in the diet, and persisted even after parenteral administration. Crystalline biotin plus folic acid concentrate prevented

the pantothenic acid content of the liver to normal with the disappearance of all symptoms. These authors discuss the possibility of folic acid and biotin playing a rôle in the bacterial synthesis of pantothenic acid or in the proper storage or utilization of pantothenic acid in the tissue.

Vitamin B₁₀ and B₁₁ concentrates⁶ proved to be inactive as sources of folic acid for the monkey, while crystalline B₆ and synthetic *L. casei* factor were quite adequate. A reversal of the lymphocyte-neutrophil ratio plus sub-optimal hemoglobinogenesis were precipitated by folic acid deficiency.

Finally, Kornberg, Daft and Sebrell¹⁰ have just shown that weanling rats become both granulocytopenic and anemic on diets devoid of adequate protein. The simultaneous addition to the diet of both *L. casei* factor and the essential amino acids was necessary for full recovery.

Prior to our own clinical studies with folic acid, extensive experimental investigations were carried out in monkeys, particularly from the standpoint of susceptibility to virus and streptococcus infections in folic acid-deficient, leukopenic states with or without anemia.

Reference to Table 1 will indicate the bone marrow and peripheral cell response to various diets (Table 2) in which the vitamin B complex is lacking in a greater or lesser degree. All monkeys developed a leukopenia after a period of 30 to 120 days on one or other of these diets, and 57% showed an accompanying anemia.⁶¹ In sharp contrast to the nutritionally normal monkey, which is quite resistant to the influenza virus and/or hemolytic streptococcus Group C, the nutritionally leukopenic monkey is extremely susceptible with a high mortality rate (Table 3).

Neutralizing humoral antibodies in re-

TABLE 1.—RED AND WHITE BLOOD CELL RESPONSES OF MACACA MULATTA ON MODIFIED VITAMIN B DEFICIENT DIETS

	Total	Developing leukopenia	Developing anemia
Goldberger diet	4	4	3
Synthetic basic diet (vitamin B-free)	4	4	3
Diet I (with supplements)	7	7	4
Diet II (with supplements)	20	20	10
	35	35 (100%)	20 (57%)

TABLE 2.—TWO DIETS LACKING IN VITAMIN B COMPLEX WITH SUPPLEMENTS

Basic Diets*	Diet No. 1	Vitamin Supplement†	
			Daily dosage (mg.)
Sucrose	68	Thiamine hydrochloride	1
Vitamin-free casein	18	Riboflavin	1
Vegetable oil	8	Pyridoxin hydrochloride	1
Salt mixture (U. S. P. No. 2)	4	Nicotinic acid amide	25
Cod liver oil (U.S.P.)	2	Calcium pantothenate	3
	100	Ascorbic acid	25
	Diet No. 2		
	Diet No. 1 with addition of:		Daily dosage (mg.)
	Choline chloride		50
	Pimelic acid		1
	Glutamine		1
	Inositol		1
	Sodium paraminobenzoate		50

* Liberal amounts of the basic diet and water were available to the animals at all times.

† Double doses of supplements suspended in water and fed by stomach tube every 2nd day. Biotin assays were done on feces of some of these animals with and without addition of biotin to the diet. Since these assays indicated synthesis of large amounts in the intestine of the monkey biotin supplements were discontinued.

sponse to the virus,⁴⁰ and specific precipitins, opsonins and antistreptolysin titers⁴¹ following the streptococcus developed in time and quantity in the nutritionally deficient monkeys as in the animals on normal diet. The most obvious difference in the 2 groups was the diminished number and inferior quality of the circulating granulocytes, and the progressive hypoplasia of the bone marrow in the absence of folic acid in the diet.

Monkey 61 (Fig. 1) is illustrative of the progressive pan-marrow hypoplasia with

events from those occurring following liver extract (Fig. 2), or when an hyperplastic, megaloblastic, maturation-arrest bone marrow is subjected to folic acid (Fig. 7) is significant. In the latter instance the peripheral leukopenia and thrombocytopenia are usually primarily myelophthisic in mechanism and the reticulocytosis precedes the granulocyte and platelet responses. In the nutritionally hypoplastic marrow in our monkeys, apparently the deficiency for granulocytopoiesis, when corrected, resulted in a re-

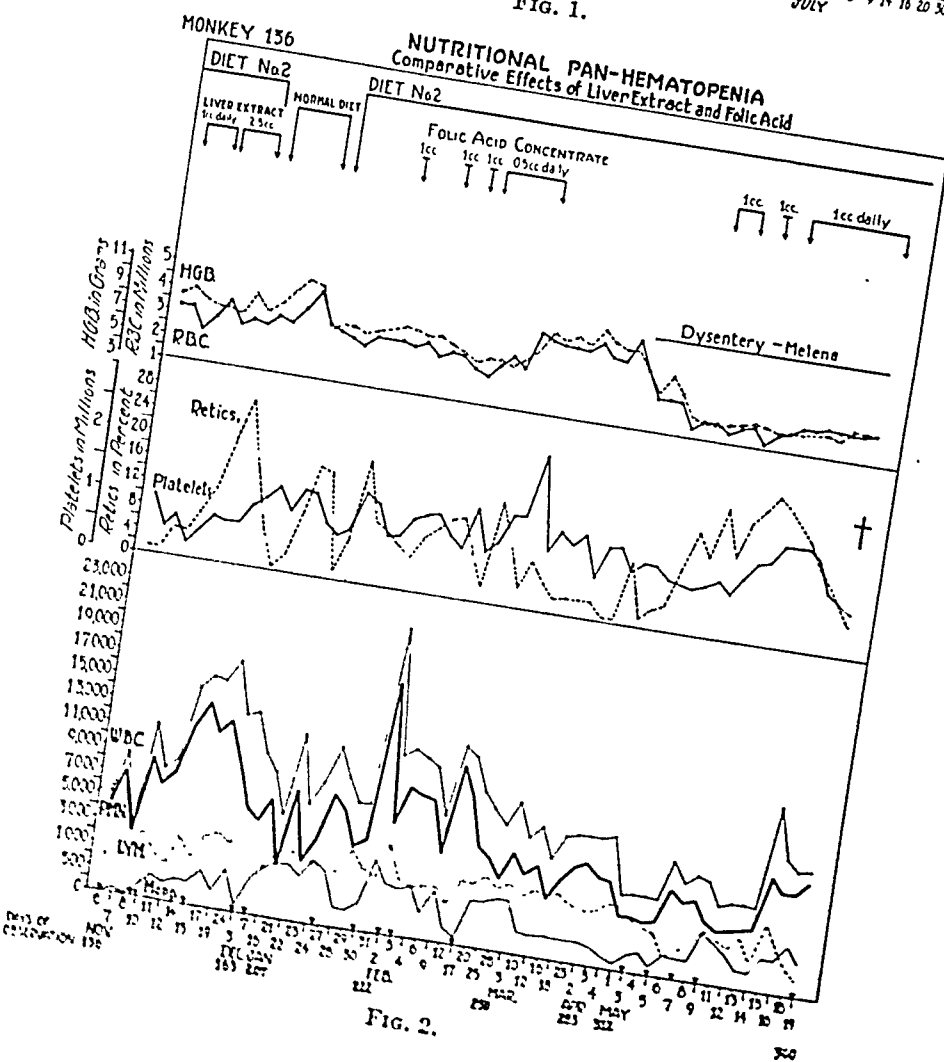
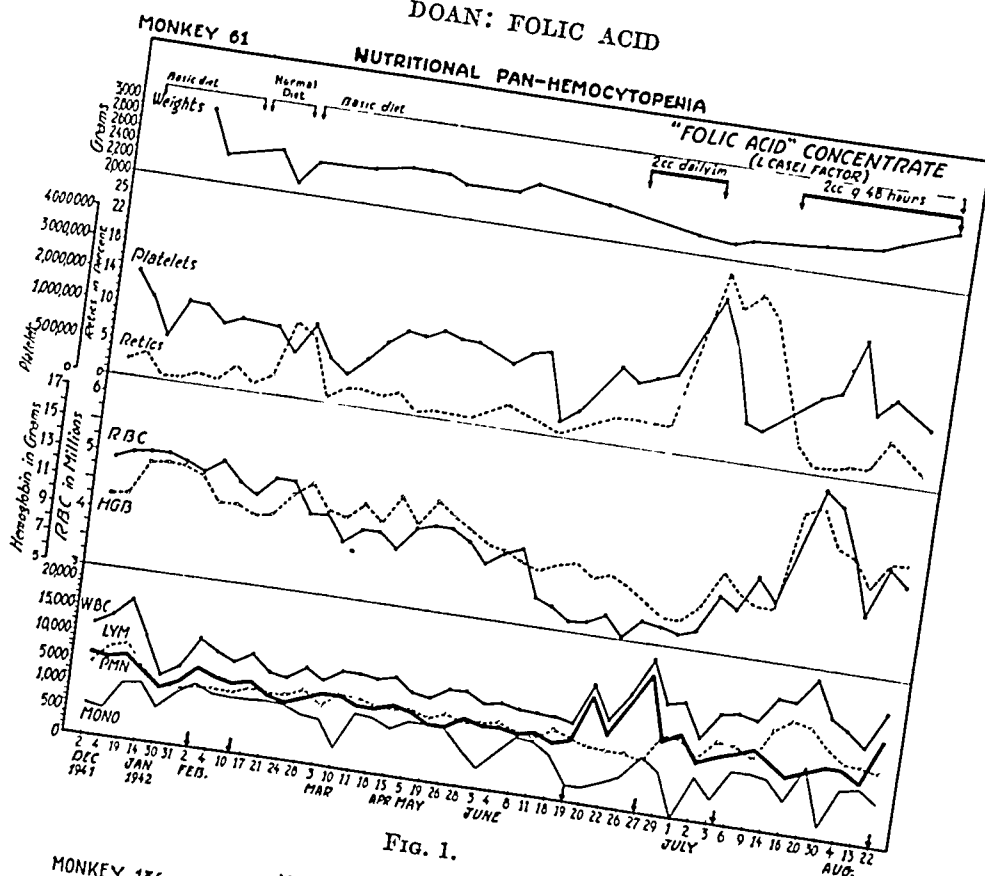
TABLE 3.—SUSCEPTIBILITY TO INFECTION OF *MACACA MULATTA* ON A MODIFIED VITAMIN A DEFICIENT DIET

I. Hemolytic streptococcus (Group C) (intranasal inoculations)					6
Streptococcus septicemia, fatal (7 to 13 days)					5 (83%)
	Diet	Survival	Findings		
M 8	600				
M 14	600	8 days	Septicemia		
M 23	I	7 days	Sepsis erysipelas		
M 55	II	7 days	Septicemia		
M 51	II	13 days	Sepsis erysipelas		
M 54	II	18 days	Sepsis erysipelas		
II. Influenza virus A (Strain PR 8) (intranasal inoculations)					7
Virus infection, fatal (2 to 11 days)					5 (71%)
	Diet	Survival	Pathology	Recovery of virus	
M 21	600	3 days	+	+	
M 27	I	2 days	+	+	
M 51	II				
M 53	II	8 days	+	—	
M 54	II				
M 56	II	11 days	+	—	
M 69	II	7 days	±	±	
Controls on Normal Optimum Diet					
III. Streptococcus hemolyticus (Group C) (intranasal inoculations)					9
Fatalities					0
Influenza virus A (Strain PR 8) (intranasal inoculations)					10
Fatalities					0
Hemolytic streptococcus followed or preceded by influenza virus					30
Fatal streptococcus septicemia (15 to 21 days)					2 (4%)
(Streptococcus preceded by virus 15 days.)					

a resulting peripheral pan-hematopenia which occurs on the unsupplemented basic diet. Immediately, within 24 hours of the administration of a relatively small dosage of folic acid concentrate, the granulocytes were increased in the circulating blood and shortly thereafter both reticulocytes and thrombocytes appeared in greater abundance, followed promptly by a steady and sustained rise in total red blood cells, hemoglobin and lymphocytes. The difference in sequence of these cellular

activation which released mature neutrophils directly and before the red blood cells have had time to respond. The reticulocytosis was greater but the granulocyte response was more delayed and less pronounced in Monkey 136 (Fig. 2) following liver extract, than when folic acid concentrate was administered.

Inasmuch as our early studies¹⁹ on the mechanism of nucleinate induced granulocytosis gave a similar prompt release of white blood cells to the circulation, when



the bone marrow was functionally capable of responding, we were interested in comparing the reaction of our leukopenic monkeys to adenylic acid *versus* folic acid. While the granulocytes rose transitorily in Monkey 1 C (Fig. 3) following each injection of adenylic acid, there was no evidence of a progressive and sustained recovery of both leukocytes and erythrocytes such as occurred on daily folic acid administration.

condition deteriorated gradually, and when influenza virus was introduced intranasally an acute episode was precipitated resulting in death 8 days later with profound leukopenia in the presence of neutralizing antibodies. Similar fatalities resulted when hemolytic streptococci were administered under the identical circumstances (Fig. 5), even though agglutinins, opsonins and antistreptolysins developed promptly.

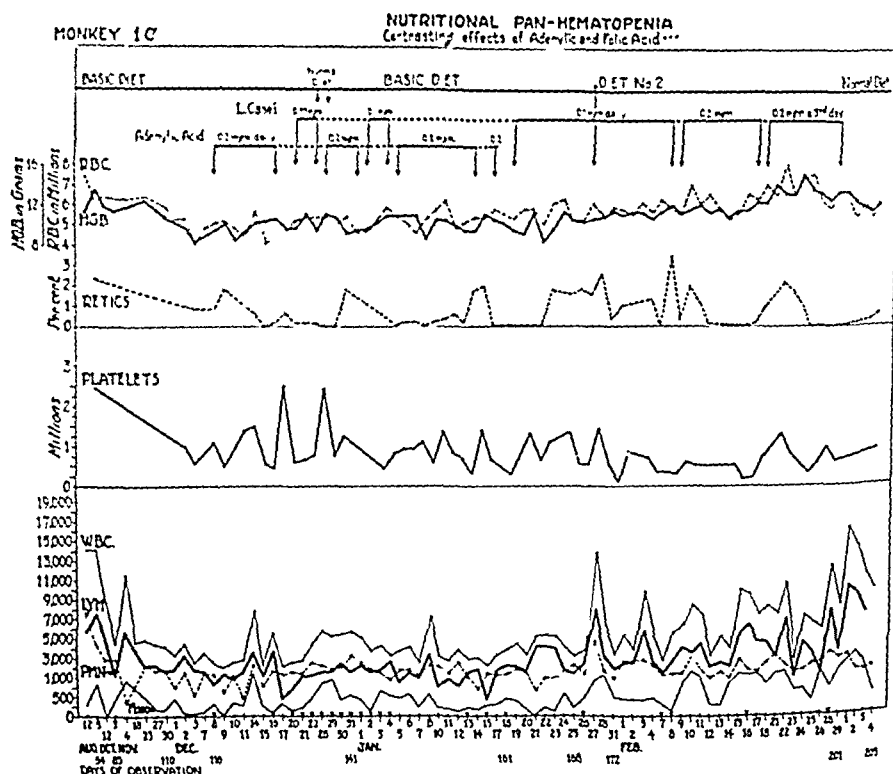
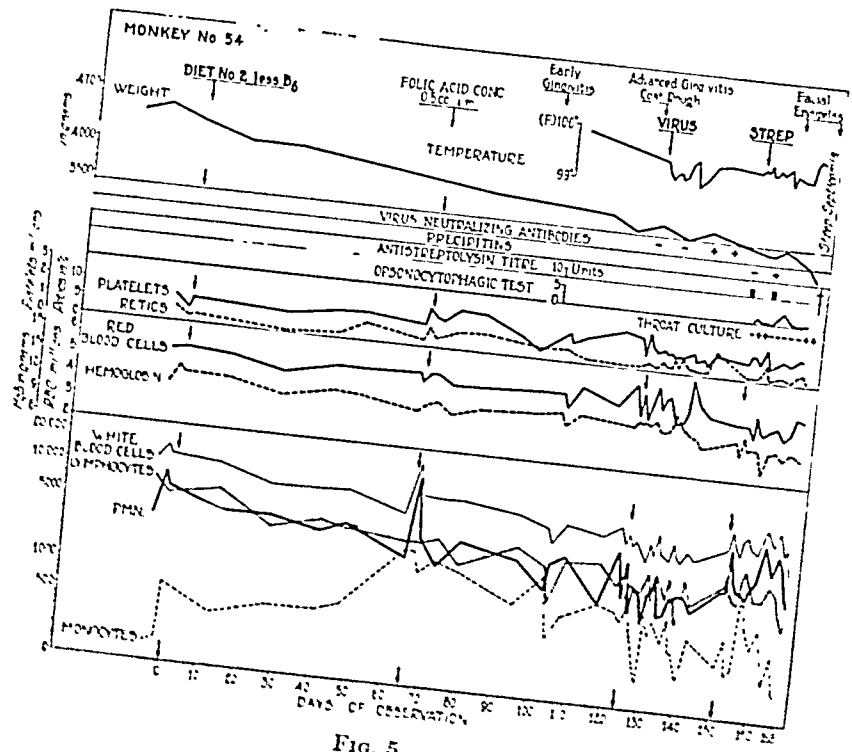
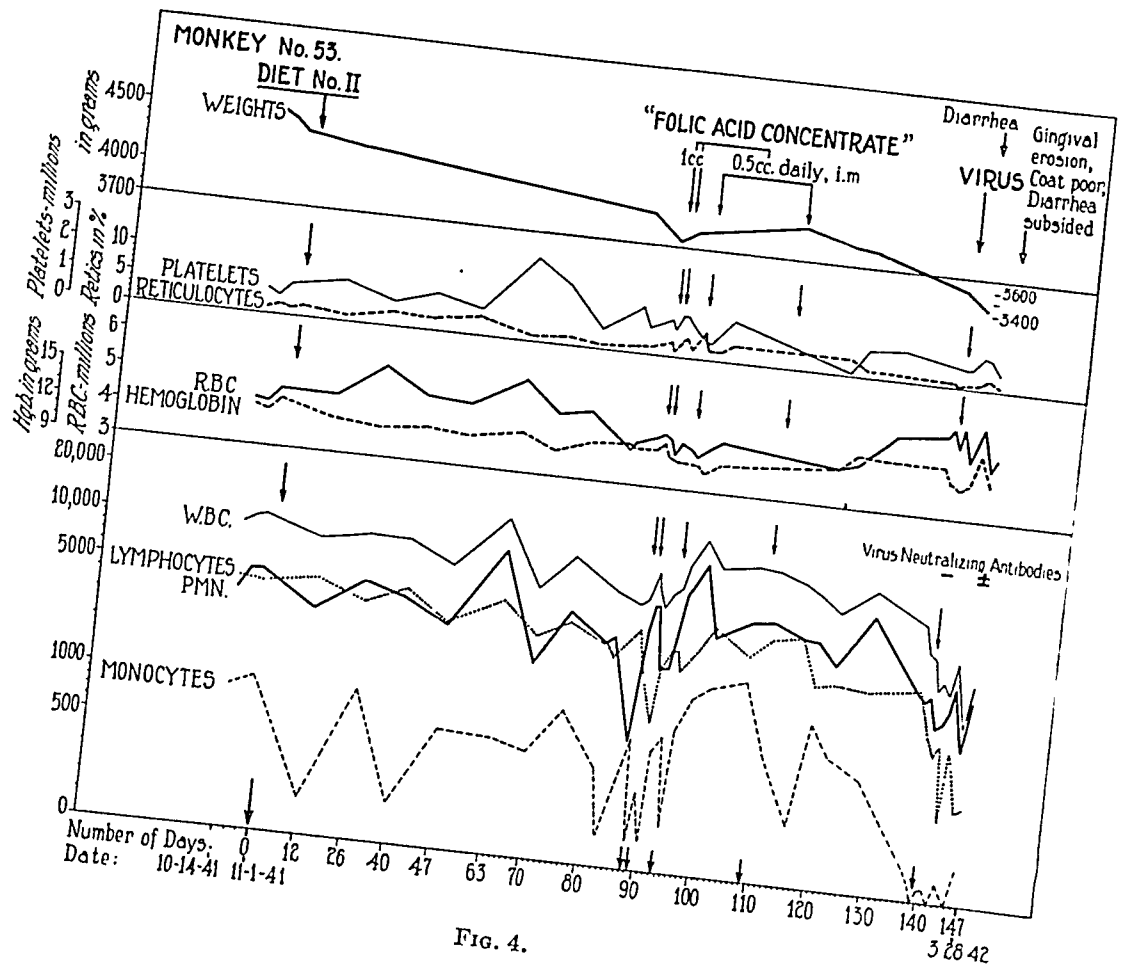


FIG. 3.

The contrast between the cytologic and clinical improvement, which followed the reavailability of folic acid in a monkey on a supplemented basic diet, II, and the fatal reaction to influenza virus infection during a leukopenic relapse is indicated in Figure 4 (M 53). Following the withholding of the folic acid concentrate after its specific and generally favorable effect on the blood cell equilibrium, appetite, weight and general appearance and activities of the animal, the cellular disequilibrium again developed, the physical

The monkeys, as their leukopenia developed on an inadequate diet, not only became abnormally susceptible to superimposed virus or/and pyogenic infections they likewise became susceptible to their own endogenous, potential pathogens more particularly those in the intestinal tract. In Table 4 are recorded the fatalities due to infection in the 35 monkeys subjected to dietary deficiency studies. It will be noted that 40% were due to spontaneous infections, the great majority succumbing to bacillary dysentery.



Thirty % did not survive influenza virus or streptococcus exposure, which represents 71 and 83 % respectively of those which received intranasal inoculations during a nutritionally induced leukopenic state. Only 1 monkey succumbed during folic acid administration (Fig. 2, M 136) in an irreversible crisis.

Another phenomenon of definite significance was observed among a few of our monkeys, which received superimposed intranasal inocula of streptococci preceded or followed by influenza virus. In

Monkey 12 (Fig. 6), some 80 days following the spontaneous recovery from an initial streptococcus-virus sequence, reinfection was accomplished with the same strains of pathogens and in the same order and dosage. After a latent period of 15 to 20 days all of the signs and symptoms of an acute diffuse glomerular nephritis developed, including anorexia, albuminuria (100 mg. %), hematuria, edema and hypertension (systolic 185 mm.; diastolic 140 mm. Hg). The high opsonic index had been sharply neutralized by the re-

TABLE 4.—MORTALITY FROM SPONTANEOUS AND INDUCED INFECTIONS (35 MONKEYS)
(MACACA MULATTE ON MODIFIED VITAMIN B DEFICIENT DIETS)

	Fatalities
I. Spontaneous <i>Sh. dysenteriae</i> (Flexner)	11
II. Spontaneous <i>Kl. pneumoniae</i>	1
III. Spontaneous hemolytic streptococcus bronchopneumonia (fatal)	1
IV. Spontaneous staphylococcus septicemia	1 (40%)
V. Experimentally induced hemolytic streptococcus (Group C)	5
VI. Experimentally induced influenza virus A (Strain PR 8)	5 (30%)
VII. Controls receiving liver extract or folic acid	1
VIII. Survivors	10 (29%)

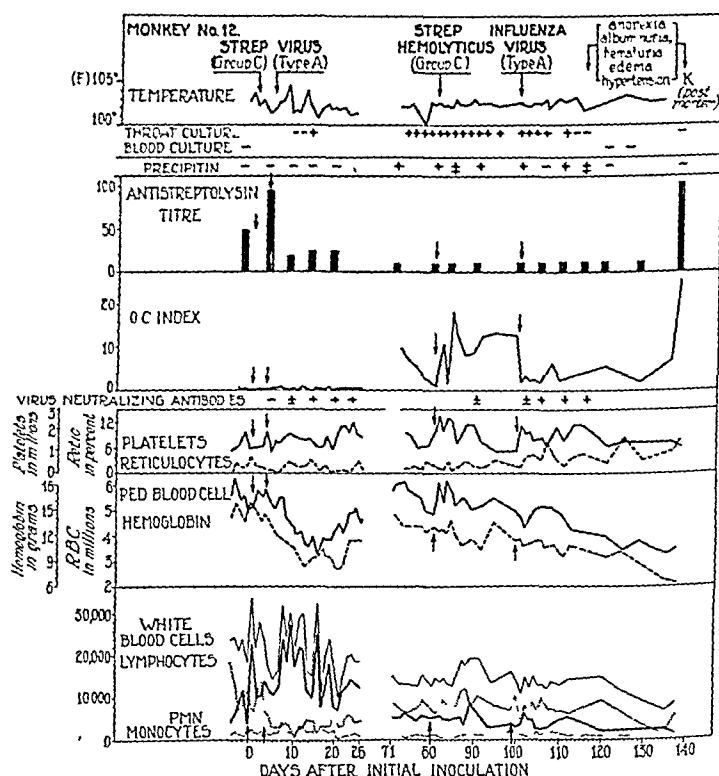


Fig. 6.

inoculation of the virus and there was a progressive anemia and leukopenia at this time, with only a slight reticulocyte response. Throat cultures were positive, but blood cultures were negative for hemolytic streptococci, and the hyperergic character of this renal episode was supported by the pathologic findings in the kidneys at postmortem. The same type of episode occurred in 2 monkeys on a deficient vitamin B diet in which spontaneous endogenous infection had occurred.

The studies of the relative susceptibility of *Macacus mulatta* monkeys, under a variety of nutritional and environmental conditions, to influenza virus and/or hemolytic streptococcus exposure *via* the nasopharynx, have once again emphasized the important rôle of the white blood cells in the body defense against infectious diseases. Furthermore, folic acid concentrate (*L. casei* factor) is at least one of the essential foods for the maintenance of the normal cellular integrity of bone marrow in the monkey, and seems to support pan-hematopoiesis more specifically and effectively than any other molecule thus far isolated and tested.

Clinical Studies with *L. casei* Factor, Synthetic. Beginning with Wills⁵⁹ discovery that marmite, an autolyzed yeast product, was as effective as liver extract in the treatment of tropical macrocytic anemia, followed by the demonstration of its efficacy in the macrocytic anemias of idiopathic steatorrhea,⁶⁵ tropical sprue,³ and, when given in sufficient dosage, in pernicious anemia,⁶² the search for chemically active hematopoietic fractions from both liver and yeast has never ceased. The preliminary attempts to apply the experimental results with folic acid concentrates derived from each of these sources to the treatment of human anemias and leukopenias were equivocal and unconvincing, despite their striking influence on the metabolism of bone marrow in experimental animals. Moore and his associates⁶¹ in 1943 attempted the assay of both crude and more highly purified

"folic acid" concentrates in typical pernicious anemia patients, always obtaining a submaximal response, which was attributed to "some extrinsic factor activity." Castle *et al.*⁴ reported negative results in relapsing pernicious anemia patients, when vitamin-free casein plus all crystalline members of the vitamin B complex, including "folic acid and folic acid concentrate" in amounts of 2.3 to 3.6 mg., were added to normal gastric juice and fed daily. Sharp *et al.*⁴⁵ obtained "an appreciable increase in the hematocrit but only slight changes in other erythropoietic phenomena" in 10 otherwise refractory anemia patients given 0.6 to 1.5 mg. of vitamin B₁₂ in yeast concentrate. Watson⁵⁷ was unable to confirm these findings in his patients with refractory anemia, when 5 mg. of *L. casei* factor were administered daily by mouth, but suggestive responses were observed in 7 patients, who had developed leukopenia following roentgen ray therapy.

In confirmation of Watson's observations, Major Perk Lee Davis^{11a} between October 1942 and July 1946, as part of a coöperative research program with Dr. Herman Hoster and our laboratory group in Columbus, observed 191 cases at the Walter Reed Hospital, Washington, D. C., diagnosed as lymphoblastomata. Included were reticulum cell sarcoma, lymphosarcoma, giant follicular lymphoblastoma, and Hodgkin's syndrome. "Complete blood and bone marrow studies revealed marked depression of both myelopoietic and erythropoietic activity. With the introduction of radiation therapy, both anemia and neutropenia became progressively more severe, necessitating daily to weekly transfusions of whole blood and/or washed red cells." Daft and Sebrell, of the National Institute of Health, were instrumental in securing, through the courtesy of Dr. Thomas Jukes of the Lederle Laboratories, sufficient quantities of folic acid to permit treatment of selected patients in this Army group with large oral doses of 75 to 150 mg.[†]i.d. "Seventeen cases, given these large doses of folic

acid without any other therapy, showed no demonstrable beneficial influence on the course of the disease. Sixty-nine cases were then treated with radiation therapy, transfusions and folic acid. When folic acid was added to the radiation therapy, it was noted that the patients withstood radiation better, with less nausea, vomiting and general depressing physical effects. The necessity for red cell or whole blood transfusions decreased in frequency to an average of one in 18 days. The average time of hospitalization was reduced from 9.8 to 5.3 months. The bone marrow on discharge showed an increase in the myeloid and erythroid elements." Davis concludes: "Folic acid is recommended as a useful adjuvant in maintaining the hematologic well-being of patients receiving massive and prolonged radiation therapy."

Zuelzer and Ogden⁶⁴ have just reported

microcytic anemia (8 cases), chronic hypoplastic anemia (2 cases), and in 1 patient each with diagnoses of Mediterranean anemia, sickle cell anemia, aplastic anemia, subacute myelogenous leukemia, and acute lymphatic leukemia, respectively.

Encouraged by the consistent and spectacular response of specifically leukopenic or pancytopenic monkeys to "folic acid concentrates" in our laboratory,^{39,61} an attempt was made, in coöperation with Dr. Spies beginning in the summer of 1943, to select certain patients at the Hillman Hospital, Birmingham, with nutritional deficiency reflected by leukopenia and mouth ulcers, and to appraise the value of *L. casei* factor, first in the form of the purified crystalline concentrate from liver, later as the synthetic molecule. Controlled peripheral blood and bone marrow studies were made, using the supravital

TABLE 5.—SUMMARY OF 28 CASES TREATED WITH *L. CASEI* FACTOR, SYNTHETIC

I. Satisfactory:		
1. Addisonian pernicious anemia		10
Virgin case		1
In relapse		3
Maintenance		3
Hypersensitive to liver		3
II. Unsatisfactory:		
2. Hypoplastic marrow		6
Primary		4
Secondary		2
3. Monocytic leukemia		5
Chronic		4
Acute		1
4. Acute influenza virus infection, with profound leukopenia		3
5. Macrocytic anemia, secondary to hepatic cirrhosis		1
6. Hemolytic anemia, acquired		1
7. Myelogenous leukemia, chronic		1
8. Lymphatic leukemia, chronic		1

12 infants under 1 year of age with severe macrocytic anemia and megaloblastic bone marrows of unknown, non-dietary deficient etiology, 9 of whom responded promptly and completely to *L. casei* factor concentrate, or synthetic. The dosage was 5 to 20 mg. per day for 8 to 21 days. Bone marrow, reticulocyte and hemoglobinogenesis curves were "virtually identical with those obtained in pernicious anemia following adequate treatment with purified liver extract." Completely negative results are reported in the anemia of prematurity (18 cases), hypochromic

technique, and both hematologic and clinical improvement, commensurate with the dosages then available, were reported.²

In November 1945, and each month since, Spies and associates⁴⁸⁻⁵¹ have presented a rapidly increasing series of patients with macrocytic anemia, who have responded dramatically and completely to parenterally and orally administered *L. casei* factor, synthetic. These cases have included nutritional deficiency *per se*, Addisonian pernicious anemia, and the macrocytic anemias of pregnancy,

pellagra and sprue, both tropical and non-tropical.

Coincidentally and independently, Darby and Jones¹¹ obtained satisfactory hematologic and clinical remissions in 2 patients with sprue, using 15 mg. of the same synthetic material, intramuscularly, daily. Moore and associates³¹ and our own group²¹ immediately confirmed, and have since paralleled and elaborated upon the reported clinical findings of the Birmingham and Nashville groups.

Table 5 summarizes the 28 patients thus far studied in our Clinic together with their respective diagnoses. Figures 7, 8 and 9 present the essential dosage require-

approximately normal cellular maturity pattern by the 10th day, the maximum reticulocyte response (26.8%) being recorded on the 15th day of therapy, after a total cumulated dosage of 30 mg. folic acid. Increasing the dose to 10 mg. daily beginning with the 16th day failed to induce a secondary reticulocyte rise, confirming the adequacy of the original dosage. On the 40th day of therapy the total red cells reached 4,000,000, hemoglobin 13 gm. from a low of 1,000,000 erythrocytes, hemoglobin 5 gm. Clinically on the 2nd day of therapy this patient volunteered that the paresthesia

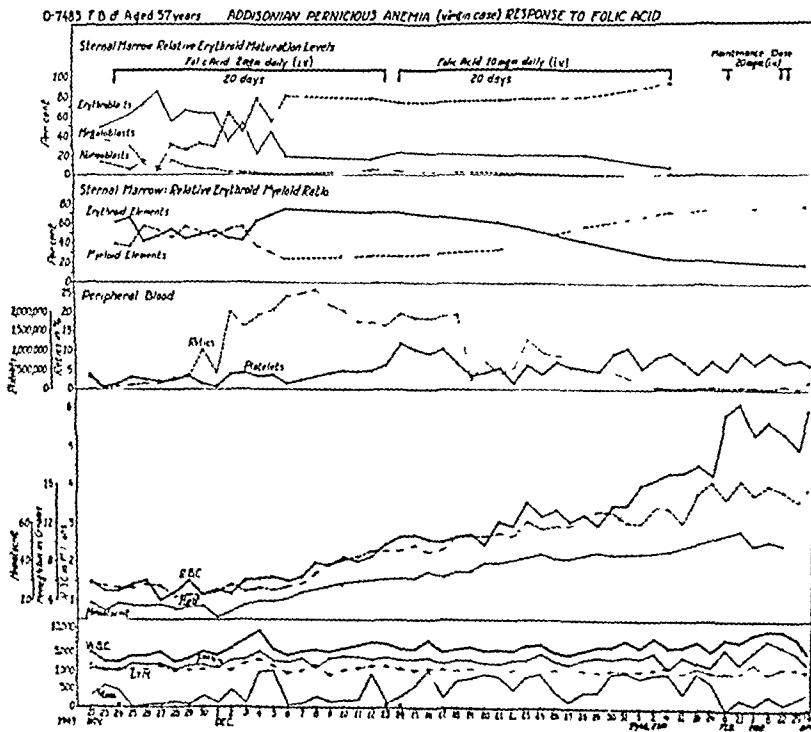


FIG. 7.

ments and bone marrow and peripheral cellular responses elicited by synthetic folic acid (*L. casei* factor) in 3 individuals with classical Addisonian pernicious anemia. The changes in the sternal bone marrow were particularly closely followed in the patient whose laboratory studies are correlated in Figure 7. Folic acid (2 mg. synthetic) was given intravenously for 20 consecutive days. A prompt and rapid maturation of the megaloblasts was observed with the restoration of an

of the extremities was definitely diminished with a very evident improvement in the subjective sense of well-being; the vibratory sense and the Romberg were objectively improved by the 21st day of therapy. These findings are identical with those observed and reported previously in patients with pernicious anemia receiving optimal parenteral liver extract therapy.²³ This patient has been maintained in hematopoietic equilibrium and abundant health on 20 mg. of synthetic folic acid at fort-

nightly intervals since the remission was established.

While 2 mg. orally of folic acid daily were insufficient to appreciably increase the peripheral red cell level after 10 days (Fig. 8) in a white female patient with pernicious anemia, in relapse, the megaloblasts in the sternal bone marrow were reduced from 45 to 24% during this period and almost immediately following the increase to 10 mg. daily by intramuscular injection, a reticulocytosis developed (22%), and 25 mg. daily, later, and 100 units of liver extract failed to induce

to this patient with pernicious anemia in relapse were suboptimal as revealed when the dosage was raised to 50 mg. with a secondary reticulocyte response.

In 1 patient (Fig. 10) a white female, aged 56, with advanced hepatic cirrhosis and ascites and with a long alcoholic history, we have observed over a period of years a chronic macrocytic anemia with moderate leukopenia resistant to therapy. The hippuric acid excretion is currently 1.49 gm., prothrombin 28%, cephalin flocculation test +++. The erythrocyte MCV is 105 μ , MCH 27, and the

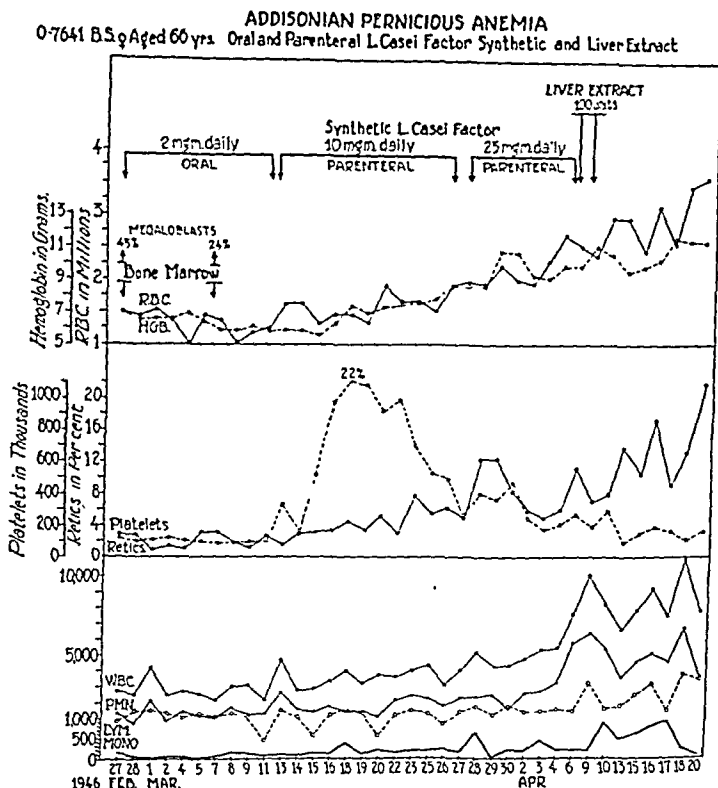


FIG. 8.

any significant secondary reticulocyte peak. The chief complaint on admission in this patient was severe angina pectoris, several attacks daily requiring nitroglycerine for relief. There have been no subjective or objective cardiac manifestations since the hemoglobin improved.

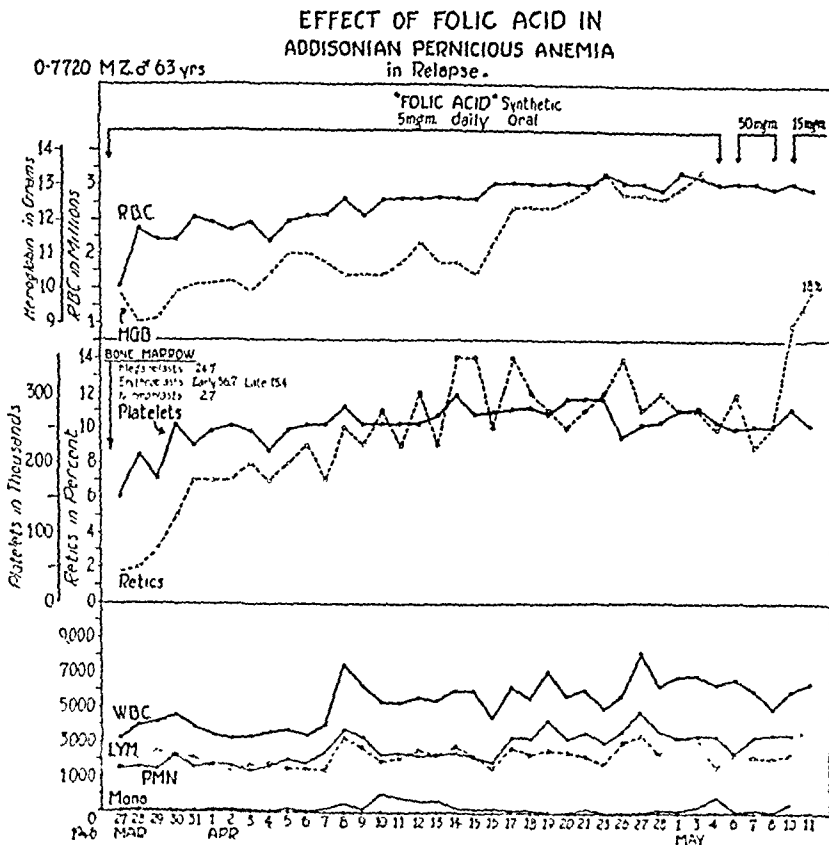
Again in Figure 9 it will be apparent that 5 mg. of folic acid given orally daily

sternal marrow is hyperplastic with an erythroid predominance showing a moderate "left shift" pattern with 5% megaloblasts, 17% early erythroblasts, 22% late erythroblasts and 58% normoblasts. Folic acid, oral and parenteral, has failed thus far to mature these elements or correct the macrocytosis as have 100 units of liver extract concentrate par-

enterally. The rôle of the liver in the utilization of folic acid may be suggested should similar macrocytic anemias with megaloblastic arrest secondary to far advanced hepatic cirrhosis continue to be refractory to this stimulus.

The combined studies have conclusively established at least 2 important and extremely significant points: (1) that *L. casci* factor contains a potent anti-anemia component completely effective in a variety of human macrocytic anemias; (2) that all of the human macrocytic anemias thus far studied, including Addisonian

day. It has been proven to be therapeutically effective when given by any route; oral, parenteral, per rectal enema (100 mg. per day).³² In several hypoplastic anemia patients, in whom we desired to give a maximum single intravenous daily dose, we found that 125 to 150 mg. represented the top tolerance level without unpleasant subjective histamine-like, vasomotor symptoms. Spies⁵⁰ reports no untoward reactions to 400 mg. daily by mouth. Maintenance dosages in patients with pernicious anemia in full remission have thus far varied in our clinic between 20



pernicious anemia, reflect a common mechanism of megaloblastic maturation arrest, not *primary* or *specific* to any one syndrome, but in every instance *secondary* to some interference in the availability of an essential nutritional complex.

The effective dosage of *L. casci* factor synthetic in a fully developed, advanced macrocytic anemia varies from patient to patient, from as little as 1 to 25 mg. per

and 40 mg. at 1 to 3 week intervals. Minor neurologic signs and symptoms in patients in relapse have responded as promptly and completely following *L. casci* factor supplements as with potent liver extract, and no progressive cord lesions have as yet been noted. In those of our patients who have developed hypersensitivity to all liver products, *L. casci* factor synthetic has been substituted in dosages

sufficient to maintain an optimum hematopoietic equilibrium with, in no instance, any intolerance or allergic manifestations to date. Idiopathic marrow hypoplasia, myelophthisic anemia, the leukopenia of virus infections, and the iron deficiency states do not, and would not be anticipated to respond (Table 5). Unfortunately, also the maturational stimulus for normal leukopoiesis has failed to affect the immature white cells in the acute

effective by mouth in relatively small volume in patients with classical pernicious anemia having diminished or absent intrinsic factor; (2) it is effective when given parenterally; and (3) its activity is not appreciably augmented by incubation with normal human gastric juice. Is it the active anti-anemia principle in liver and liver extract? It would seem unlikely, since: (1) the minimum effective dose is 1 mg., usually 4 to 10 mg. daily, while

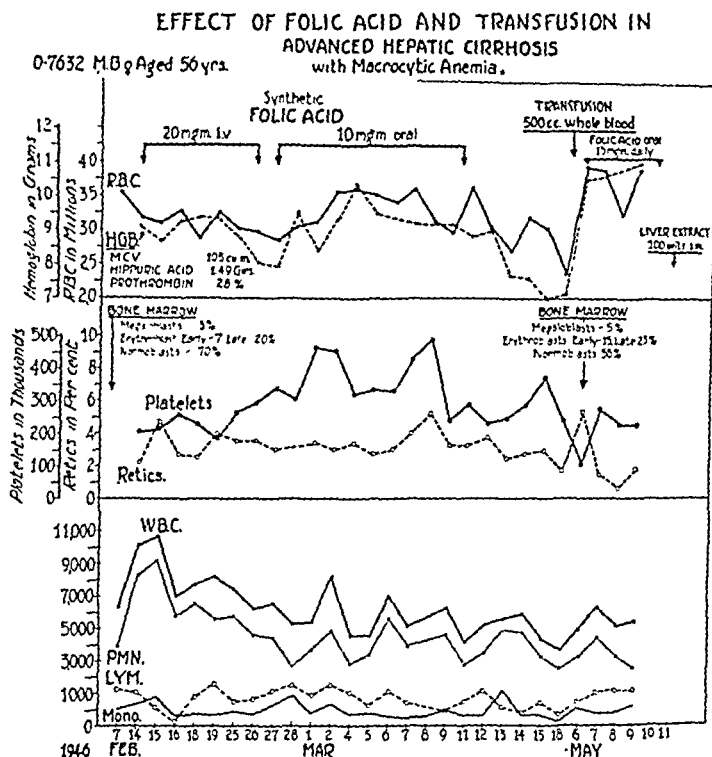


FIG. 10.

and subacute leukemias, in a limited experience, but a principle has been established for further elaboration and exploration in this area.

There is naturally much speculation as to the precise rôle played by *L. casei* factor in erythropoiesis and pan-marrow hematopoiesis. With the exact chemical formula still unrevealed,* analogies still may be drawn between this factor and the other known, essential marrow stimulating agents. Does it act as "extrinsic factor?" Probably not, since: (1) it is

potent purified fractions of liver "containing only 0.035 to 0.7 mg. of material per injection"⁵⁴ have produced adequate hematologic effects; (2) *L. casei* factor assay has revealed 0.02 to 0.5 mg. only per U.S.P. unit of liver extract;⁵ (3) the minimal effective oral dose approaches the minimal effective parenteral dose; (4) no evidence of antigenic sensitization has yet developed following folic acid. It must be remembered, however, that, with the recognition of *L. casei* factor conjugates reducible by enzymatic action with the

* "Pteroylglutamic Acid," Science, 103, 6, 1946 (May 31).

release of "free" factor, the mere presence in food or gastro-intestinal tract of "folic acid" need not mean it is in a utilizable form. Moore³¹ suggests that the intrinsic factor of Castle may be concerned with the release in man of the *L. casei* factor from its combined form. Spies⁵⁰ offers the hypothesis that the anti-anemic factor present in liver and yeast is either a much larger molecule than *L. casei* factor, or represents a more potent or effective combination of chemical substances per unit volume. The precise explanation awaits further evidence.

Still more recently Spies⁵¹ has reported the erythropoietic effectiveness of 2, 4-dihydroxy-5-methyl-pyrimidine synthetic (thymine), in dosages of 4.5 to 20 gm., oral administration, daily with reticulocyte response and red blood cell recovery in human macrocytic anemia. Thymine is a normal constituent of body cells and has been shown to support the growth of *L. casei*. While the quantitative requirements are much greater for equivalent bone marrow effects, it would seem probable that this, too, represents one more link in the same chain of evidence, which is being currently accumulated.

Conclusion. In analyzing the increasing volume and variety of both clinical

and experimental studies with "folic acid," as derived from spinach, liver, yeast, other natural sources, and now, the synthetic *L. casei* factor, it becomes increasingly apparent that we are dealing with one of the more fundamental molecules essential to the normal metabolism of all cell types in marrow, and in young, actively growing cells and tissues generally, perhaps. In the absence of "sufficient" *L. casei* factor due to extrinsic dietary deprivation, or with intrinsic interference in absorption or utilization, a more or less marked, specific or general, cellular inadequacy develops in mammalian bone marrow with the appearance of signs and symptoms associated with, and dependent upon, the extent and specificity of the individual's needs. Many other factors, including infections, condition and modify the relative clinical stability or instability of each human organism. The alert physician must, therefore, recognize these variables, and exert the maximum of ingenuity in his search for the "balance of power" among the known or knowable factors in the equation, and when this is done, more often than not, the problem can, and will, be solved.

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PRIMARY SYSTEMIC AMYLOIDOSIS: JAUNDICE AS A RARE ACCOMPANIMENT

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PRIMARY systemic amyloidosis is a rare pathologic entity and, although first described by Wilks in the Guy's Hospital Report for 1856 as mentioned by Dillon and Evans,⁴ was only noted 24 more times in the literature up until 1939 according to Koletsky and Stecher.⁸ Recently Lindsay and Knorp¹⁰ in reviewing the literature noted 16 more cases, including 1 of their own. Eight of these had appeared before 1939. Since their paper Brown and Seltzer,³ and Golden⁶ have added 2 others.

At the Montefiore Hospital, New York City, there have been 3 cases in the last 3 years. Perla and Gross¹⁴ in 1935 originally reported 3 others from this institution, but in retrospect their second case was probably secondary to chronic rheumatoid arthritis. Of the recent 3 cases, the first was a case of primary amyloidosis of the heart, the second of the liver, spleen, kidneys, gastro-intestinal tract and blood-vessels, and the third of the cardiac and striated muscle and of the vessels and lungs. Only the second will be discussed here because of the very rare finding of jaundice of many months duration in man with hepatic amyloidosis and a large gastric ulcer.

Most authors, including Moschcowitz,¹² have stated that amyloidosis of the liver is either rarely or never accompanied by jaundice. Pearlman¹³ and Rosenblatt¹⁷ in a large series of cases make no mention of its presence. In a study of hepatic function in this disorder, Tiber, Pearlman and Cohen²⁰ noted no icterus in 30 cases personally observed and only 1 case of transient jaundice in the records of 100 patients with severe liver amyloidosis.

Bannick, Berkman and Beaver,¹ however reported a case of a 44 year old man who 1 week before death developed obstructive jaundice and ascites and at autopsy had only generalized amyloidosis of the liver, spleen and other organs. There was extensive atrophy of the hepatic cords between which there was an accumulation of amyloid for which there was no apparent cause. Their third case had a bilirubin of 1.7 mg. with liver amyloidosis and a stomach carcinoma. Theirs were apparently the first such cases reported in the literature, but there was no discussion of the pathogenesis of the jaundice.

Recently Spain and Riley¹⁸ reported another case. They reviewed 50 cases of liver amyloidosis and only noted 1 with jaundice. This was a 29 year old female with chronic pulmonary tuberculosis who had extensive liver amyloidosis and developed clinical jaundice 1 week before death. Unfortunately no bilirubin determination was done, although before that time it had been normal, as was the cephalin flocculation test. The alkaline phosphatase, though, had been elevated to 20 Bodansky units. It was their interpretation, after reviewing the slides of 35 other amyloid livers without jaundice and with less extensive amyloid replacement, that the icterus was due to both an actual numerical insufficiency of liver cells and a blockage of bile canaliculi.

The other unusual feature in our case was a large gastric ulcer. Gastro-intestinal involvement by amyloid is not uncommon and symptoms such as diarrhea and melena are often found in this condition. Golden⁶ noted that Lubarsch in 1929 described a case of amyloidosis of the

stomach with pyloric ulcerations and hematemesis. His case had no free acid and the amyloid involved the muscularis and vessels. Koletsky and Stecher⁸ quote Steinhaus in 1902 as having a case of amyloidosis with gastro-intestinal bleeding and involvement of the stomach. One of Perla and Gross¹⁴ cases had severe duodenal ulcerations. Recently Golden⁶ reported a case of a 66 year old colored female who had had ulcer symptoms for many years. Examination of the stomach after gastrectomy revealed amyloidosis of the entire wall and 2 superficial ulcers near the pylorus supplied by amyloid infiltrated vessels. Lindsay and Knorp's¹⁰ case had typical ulcer symptoms and at autopsy had a lesser curvature ulcer which they thought was on an amyloid basis.

Case Report. (M. S., No. 37542) This was the first Montefiore Hospital admission of a 44 year old male welder who had begun to complain of intermittent constipation and right upper quadrant pain in May 1943. Six months later he was admitted to another hospital because of a 15 pound weight loss and icterus. Physical examination at that time revealed only jaundice, but a gastro-intestinal X-ray series demonstrated partial obstruction at the hepatic flexure suggestive of neoplasm. At laparotomy only adhesions about this portion of the colon were found. The gall bladder emptied easily and contained no stones, but the liver was enlarged and peculiarly yellowish. A biopsy taken was subsequently reported as showing cloudy swelling, fatty infiltration, and dilated sinusoids with precipitated bile. Jaundice increased postoperatively and after a course complicated by a sulfonamide-responsive pneumonia the patient was discharged on a low fat diet and given injections of liver extract for 10 weeks. Seven months later, in May 1944, he was admitted to Montefiore Hospital, complaining of progressive weakness, weight loss, pruritus and abdominal distention. He also had begun to note right upper quadrant and epigastric pains following meals and relieved by Seidlitz powders. Edema and ascites had appeared 1 week previously.

Physical examination showed him to be poorly nourished and markedly jaundiced. His heart and lungs were normal. Blood pressure was 100/60. Liver and spleen were both markedly enlarged and ascites and edema were present.

Laboratory Data. The hemoglobin was 13 gm., with 4.5 million red cells, hematocrit 35.5, white count 13,600 (80% neutrophils, 12% lymphocytes, 2% basophils, 1% monocytes). Urine always showed from a trace to 2+ protein, was positive for bile and a minimal amount of urobilinogen, concentrated to 1.024 in casual specimens, and had occasional red blood cells, granular and hyaline casts. The stool gave a 4+ benzidine test but was light brown in color. Gastric analysis showed blood, free acid up to 11 units and total up to 22. Blood urea nitrogen was 12.8 mg. per 100 cc., sugar 84 mg., total protein 6.6 gm. with an albumin of 3.9 and a globulin of 2.7. The euglobulin was 0.7, pseudoglobulin I 1.2, pseudoglobulin II 0.8. Blood calcium was 10.7 mg., phosphorus 3.9 mg., bilirubin 16.3 mg., the van den Bergh reaction was direct, icterus index 71, total cholesterol 512 mg. with an ester fraction of only 112 mg., alkaline phosphatase 36.6 Bodansky units, cephalin flocculation negative, prothrombin time 23 seconds on whole blood and 63 on a dilution of 1 to 8. The Extol-Rose 2-dose glucose tolerance test showed a fasting blood sugar of 83 mg., 95 in 30 minutes, and 102 in 1 hour. Urea clearance was 73%. Hippuric acid excretion was 2.39 gm. in 4 hours. Urine was negative for melanin. The 24 hour 17-ketosteroid excretion was markedly depressed to 0.9 mg. The test for syphilis was negative. The abdominal fluid had a total protein of 2.6 gm. per 100 cc. Electrocardiogram showed left axis deviation. Roentgen ray of the chest demonstrated basal atelectasis, elevation of the diaphragm, and dorsal kyphoscoliosis. The gastro-intestinal Roentgen series showed a large gastric ulcer on the lesser curvature which was confirmed by gastroscopy. Thorotrast was not visualized in either liver or spleen by Roentgen ray after intravenous injection. Barium enema and long bone Roentgen rays were normal. No Congo red test was done.

Course. While no definite diagnosis was achieved, it was suspected that the patient

had a gastric malignancy with liver metastases. Supportive therapy did little to affect the progressive downhill course, and the patient died 1½ months after admission.

AUTOPSY. The anatomic diagnoses were: primary amyloidosis of the liver, spleen, kidneys, adrenals, heart, pancreas, lymph nodes, testes, small arteries and veins; thrombosis of the portal, splenic and superior mesenteric veins; thrombi in small branches of the pulmonary arteries and veins; jaundice; ascites; edema of the lower extremities; hypertrophy and dilatation of the heart; slight fatty degeneration of the heart; congestion and edema of the lungs; bronchopneumonia; large chronic ulcer of lesser curvature of stomach.

All of the above-mentioned organs showed the typical gross amyloid stain with iodine. The liver weighed 5900 gm. and was large, firm, friable and pale yellowish brown in color. On section the cut surface had a dull glistening appearance. The liver lobules were distorted and there were small areas of hemorrhage. The gall bladder contained no stones, had patent ducts, and bile was easily expressed into the duodenum. The spleen weighed 1200 gm. and was also yellowish brown with hemorrhagic areas. The stomach was markedly dilated with absent rugæ. There was a large gastric ulcer, 5 cm. in diameter, on the lesser curvature.

Microscopic examination of the liver revealed extreme amyloidosis with obliteration of most of the sinusoids and replacement of most of the parenchyma. Only scattered remnants of liver cords or small bile ducts remained in places. The small arteries and arterioles showed marked amyloid thickening with narrowing of the lumen. There was also an organizing thrombus in a large branch of the portal vein. In a very few foci there were bile thrombi present. Congo red stain on liver sections was positive. Sudan III stain showed fatty degeneration of remaining liver cords.

The stomach section was taken at the edge of a deep ulcer which had extended through all the layers of the wall. It was of usual chronic type with well-formed granulation tissue, liver adhesions, foci of hemorrhage, and infiltration by plasma cells, lymphocytes and eosinophils. Many of the

arteries in the ulcer base showed subintimal thickening and thrombi. There was present what was probably amyloid deposition about some of the capillaries in the mucosa and in the walls of small arteries in the submucosa. The muscle bundles in the muscular layers were separated by fibrous tissue or amyloid.

The rest of the autopsy revealed generalized amyloidosis as evidenced by iodine and Congo red stains as well as the microscopic picture.

On reviewing the original biopsy taken at the other institution it was found that it too demonstrated extensive amyloidosis and fatty degeneration.

Discussion. The overall picture of cachexia, hepatosplenomegaly, obstructive jaundice and melena naturally suggested a diagnosis of gastro-intestinal malignancy with liver metastases and when this was not confirmed at autopsy it came as a complete surprise.

It is generally stated in the literature⁸ that primary amyloidosis affects mainly mesodermal tissues, the gastro-intestinal tract, smooth, skeletal and cardiac muscles; that it stains atypically, and that it is unassociated with any known etiologic agent. However, as in our case, many^{8,12,15} have noted amyloidosis of primary or idiopathic origin distributed in the parenchymal organs as is more usual in the secondary type disease.

The rarity of jaundice in amyloidosis has been well emphasized. Hamilton,⁷ Drinker⁵ and others² have never seen amyloidosis result from welding, although transient jaundice occurred on at least 1 occasion. Among 2260 autopsy protocols at Montefiore, there were 102 cases of amyloid disease, none of which had jaundice. Lichtman⁹ goes so far as to say that when jaundice does occur it is due to concomitant cirrhosis or gumma.

Why jaundice occurred in our case, as well as in Bannick's and in Spain's, is not clear. The latter's contention, that it is due to bile canaliculi obstruction plus actual cellular insufficiency, is supported by the paucity of liver cells in our case, the pressure atrophy, bile thrombi and the

enormous weight of the liver (5900 gm.) in comparison with the weights of the 102 livers without jaundice (none over 3300 gm.). Unfortunately, however, this concept does not explain the absence of jaundice in similar cases, since other amyloid livers noted at Montefiore had as marked a numerical insufficiency of cells microscopically.¹⁶

The quantitative source of bilirubin (hemoglobin turnover) as well as the activity of the manufacturing cells would seem to be of importance in the pathogenesis of jaundice. Many of these patients are profoundly anemic so that the low hemoglobin as a limiting source of bilirubin, when coupled with a deficiency of reticulo-endothelial cells which ordinarily form bilirubin, might explain the absence of jaundice. Our patient, in contrast to most cases of amyloidosis, had a practically normal hemoglobin. On the other hand, his reticulo-endothelial system was seriously depleted as shown by microscopic replacement of lymph nodes and spleen with amyloid and the absence of Küpffer cells in the liver. Thorotrast visualization according to the method of Yater,²² *et al.* failed. The interpretation of this might well be that there were not enough reticulo-endothelial cells present to take up thorotrast in sufficient concentration to cause Roentgen ray visualization. Apparently, then, the presence or absence of jaundice in most cases of amyloidosis may depend upon a balance between a sufficient source of bilirubin, active reticulo-endothelial cells to form it, and bile canaliculi obstruction. Most often there is no jaundice because of anemia, destroyed reticulo-endothelial cells and insufficient amyloid obstruction.

It is interesting to note the dissociation in liver function studies in our case. The

elevations of bilirubin, alkaline phosphatase and total cholesterol all pointed to obstructive jaundice, while the diminished hippuric acid synthesis, low cholesterol ester and prolonged prothrombin time suggested parenchymatous disease. Despite the latter, the cephalin flocculation test, one of the most sensitive indicators of hepatocellular dysfunction, was negative. Perhaps this speaks for an insufficiency of gamma globulin as suggested by Moore *et al.*¹¹ to explain negative tests.

Gastro-intestinal infiltration by amyloid is generally extensive in so-called primary amyloidosis, both in the muscularis and nutrient blood-vessels. When the latter are severely affected, mucosal ulceration might conceivably appear. In our case it was not too obvious and the ulcer could just as well have been of the usual idiopathic type.

It is quite obvious from a review of the literature that the clinical recognition of primary amyloidosis is even rarer than the pathologic entity. Many have emphasized the possibility of amyloidosis in obscure hepatosplenomegalies, cases with macroglossia,²¹ and anemia. Now, perhaps, unexplained jaundice should also be viewed with suspicion and a Congo red test¹⁹ performed. Unfortunately, especially in cases of primary origin, this may be negative and the diagnosis can then only be made on biopsy.

Summary. 1. A case of primary amyloidosis with jaundice and a gastric ulcer is presented.

2. The rarity of jaundice in amyloid disease of the liver is emphasized by the few instances (4 cases) in which that association has been reported.

3. The possible pathogenesis of jaundice and gastric ulcer in amyloidosis is discussed.

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POSITIVE REACTIONS TO THE KAHN TEST FOR SYPHILIS—THEIR
INCIDENCE AND MEANING IN HEALTHY AMERICAN MEN

A SURVEY OF 82,070 U. S. MARITIME SERVICE ENROLLEES

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WHEN a healthy American male exhibits a positive serologic test for syphilis, what is the likelihood of his having the disease? The present report presents one answer to this question in terms of experiences with the Kahn test at a large semi-military training station. Summarized are the serologic findings and their clinical interpretations in a well-studied series of 82,070 male Maritime Service enrollees. The quota of false positives and sero-positive syphilis, congenital as well as acquired, may be assumed to be fairly typical of conditions existing in the comparable adult male population of this country, within the limits of age, race and geographic distribution.

World War II has been responsible for the performance of millions of serologic tests for syphilis on Selective Service registrants and recruits of the Army, Navy and other fighting forces. A number of statistical tabulations of the sero-positive reactors in various states and fractions of states^{2,4,6,7,10,12,14,15,18,19} are already on record. The largest series as yet reported has been that of Vonderlehr and Usilton,¹⁸ who in 1942 collected and tabulated nearly 2,000,000 serologic reports from Selective Service sources. The prevalence of syphilis for the whole country over the age period, 21 through 35, was calculated as 45.3 per 1000. This figure was based exclusively upon positive and doubtful blood tests. The true rate was then estimated as being closer to 47.7 per 1000, to allow for the fact that not all syphilitic cases give positive blood responses. The prevalence of false positives was not evaluated. Lowest rates for syphilis were

found for these areas in which most of our own subjects were recruited, namely, New England, the North Central, and the Middle Atlantic groups of states.

That serology *per se* cannot be depended upon to pick out every case of syphilis, was well brought out in the 1941 evaluation of State Laboratories.¹¹ The criterion of skill, as chosen, was the ability to demonstrate positive reactions in blood from known syphilitic subjects. In success these laboratories ranged from 61.3 to 81.3% with the Kahn Standard test; from 65.6 to 83.9% with complement fixation techniques; and from 70.3 to 83.5% with the Kline Diagnostic precipitation test. The existence of a sero-negative fraction within the total population must be kept in mind when considering statistics gathered from serologic sources.

The present survey differs from most of the others. More attention has been devoted to distinguishing between true and false positive reactions and between acquired and congenital syphilis. All interpretations of sero-positivity are based upon careful clinical work-ups by a well-organized venereal disease clinic.

Modern syphilologists are trained to view every sero-positive individual with the highest suspicion. This aggressive approach embodies the accumulated tradition of American medical thought, and through the years has proved highly efficient in stimulating the detection of latent cases needing treatment. Since the outbreak of World War II, however, the awareness is growing that diverse other conditions often give rise to non-syphilitic positive responses. As a conse-

quence, it is now being counselled that sero-positive individuals who are negative clinically should not receive antisymphilitic treatment unless their blood reactions remain constant for a minimum of several months without drop in titer; and then only when all other known causes of false positive reactions have been painstakingly excluded.¹⁷

Blood reactions positive from causes other than syphilis fall into 2 logical divisions: those due to faulty technique in collection or testing of the specimens (usually called "technical false" positives); and those due to non-symphilitic reactive substances actually present in the serum (usually called "biological false" or "non-specific" positives).

Positives resulting from faults in collection or testing originate from mislabelling, bacterial contamination, hemolysis due to improper collection and storage, faulty controls, inaccurate readings, defective reagents, the addition of oxalate or citrate, clerical mistakes, and so forth. It has been customary to ascribe to such causes those instances in which a positive reaction of strong or weak intensity is encountered only once. Such usage merits serious reconsideration in the light of recent observations that the weak sero-positivity associated with non-symphilitic ailments may last but 1 or 2 weeks.

Positive reactions of non-symphilitic origin may be associated with any one of a wide variety of infectious diseases—among which may be mentioned yaws, leprosy, malaria, infectious mononucleosis, measles, upper respiratory infections, vaccinia, and the bacterial and "viral" pneumonias. Kolmer,⁸ Davis,³ and Beerman¹ have recently re-evaluated the immunologic literature on this subject. "Attempts to find consistent empirical physico-chemical differences between syphilitic and false positive sera have thus far failed."³

The first large-scale analysis of the incidence of non-symphilitic positive reactors among otherwise normal individuals was that of Eagle.⁵ In 1941 Eagle reported on screening tests of 40,545 male and

female students in 15 American colleges. The initial testing uncovered 190 positive specimens, but 128 could not be confirmed by a second blood examination. These 128 were classified arbitrarily as due to laboratory errors in technique, possibly without significant justification. The interval between tests was not stated.

Of the 62 students whose responses remained persistently positive or doubtful, 26 were found to be either actively syphilitic or recipients of previous antisymphilitic treatment. Remaining were 36 "doubtful" students with no clinical signs or known history of syphilitic infection, a prevalence of 1 in 1125 (0.09%). Of these, 15 came from 7 schools (5783, total enrollment) which had 11 cases of syphilis. At the other extreme, in 10 schools (11,287, total enrollment), with no proved case of syphilis, only 1 student had a positive serologic test.

To these relationships, the author applied the coefficient of correlation and derived a "corrected" prevalence for biologic false positive reactions of "less than 1 in 4000." This "corrected" estimate has received wide acceptance in text-books and reviews, but the methodology employed may be subjected to criticism. Not all 36 members of the "doubtful" group were followed for a 3 to 6 month period to see what happened to their serologic reactions. The largest college had 6 syphilis and 12 "doubtful" cases, an incidence greatly different from that of the other schools. If this outlying datum had been excluded from the calculations, only the slightest relationship between the 2 kinds of responses would have been demonstrable. Nor does a given correlation coefficient between 2 serologic variables indicate that a definite percentage of 1 belongs with the other. In fact, there is a grave question, statistically speaking, whether the coefficient of correlation technique is applicable to such data. To report the initial prevalence value as 1 in 1125 would seem more advisable than to introduce this corrective computation of doubtful validity.

Mention may be made also of the report of Stokes *et al.*¹⁶ on the serology findings among 210,261 Red Cross donors tested at the Philadelphia Blood Donor Center in 1944. Only 489 (0.23%) gave definite positive reactions to serologic tests for syphilis, of whom 59.5% were adjudged, after exhaustive study, to be of a non-syphilitic nature. The prevalence of such non-syphilitic positive reactions, therefore, averages 1 in 708 in this series.

Procedure. The 82,070 subjects of our study were recruited in the northeastern United States, in the segment extending from New England down to and including North Carolina and as far west as the Mississippi River. The series comprised every basic-training enrollee reporting between June 1, 1943, and July 1, 1945. Approximately 60% were between 16 to 20 years of age, and another 30% were between 21 and 30; their age distribution is indicated in Tables 2 and 3. They came from every economic and social level. Nearly all were white. Preliminary serologic testings at the time of their enlistments were not done, and the majority had never previously been given a "blood test."

Since the Kahn test is utilized by the United States Navy and procedures within the Maritime Service are in general patterned after those of the Navy, the Kahn test was adopted for the serologic screening of these men for syphilis. Conclusions drawn with regard to sero-positive reactions are, therefore, based upon the results of this test.

Blood specimens were taken within 48 hours of arrival. The specimens were subjected to the Kahn Standard test, or to the Kahn Presumptive test, followed by the Standard test on all suspicious and positive reactions. These tests were done, as a rule, on the 1st or 2nd days after collection, although uncontrollable factors at times made delays necessary. Whenever the reaction was positive, doubtful, or difficult to read, the Kahn test was repeated. At the same time a duplicate specimen of blood was sent to

the Venereal Disease Research Laboratory of the U. S. P. H. S., U. S. Marine Hospital, Staten Island. There the sera was checked by a serologic "battery" of 7 precipitin and complement fixation tests—the Kahn Standard, the Kolmer Complement Fixation, the Kline Diagnostic, the Kline Exclusion, the Eagle Flocculation, the Hinton Flocculation and the Mazzini Flocculation tests.

The serologic "battery" furnishes a broader picture of the serum reactivity patterns than any single test procedure. Sera with a strong content of reactive substances are positive to all tests. Positive sera from treated and non-syphilitic individuals often give weak, doubtful, or negative readings with some of the tests and negative findings with others. Mahoney⁹ has pointed out, and our results also indicate, that the distribution patterns from different individuals may be diverse and non-uniform.

The reliability of the Kahn technique in our laboratory was controlled by comparisons of all positive reports with the results of parallel Kahn tests made by the Venereal Disease Research Laboratory. Apart from minor variations in degree of positivity in borderline sera, the reports from the 2 laboratories seldom conflicted.

Every subject who showed any positivity with the repeat Kahn or with any of the battery components was studied clinically by a trained syphilologist, to establish the presence or absence of syphilis.

Terminology. Simple and unconfusing terms have been chosen for classifying the positive reactions. "Positive reactions of questionable reliability" seemed an appropriate designation when sero-positivity was found once only, for the reason that it is objective and non-interpretative. To call such instances "technical false positives," as is often done, would be shutting one's eyes to the possibility that serologically reactive substances may have been transitorily present only at the time of the first test. Furthermore, the term "technical" implies an error on the part of technician or laboratory, whereas, in

actual fact, the hemolysis which gives rise to most extrasomatic positive reactions originates in the physician's venepuncture.

"Non-syphilitic positive reactions" seemed a good descriptive term for the repeated finding of sero-positivity in individuals, when diagnostic study could not establish the existence of syphilis. Such reactions are at times referred to, more

syphilitic. Those who show a persistently positive test are regarded as syphilitic, and all others still showing conflicting serologic reactions are subjected to further observation and study."

In the study of these cases, syphilis and the other diseases known to evoke serologic responses were searched for carefully when recording the history and in the

TABLE 1.—DIAGNOSTIC CLASSIFICATION OF POSITIVE SEROLOGIC REACTIONS (WITH THE STANDARD KAHN TEST) SHOWN BY 82,070 ENROLLEES

Diagnosis	No.	All enrollees (%)	Adequately studied positive reactions (%)
1. Single positives of questionable reliability . . .	257	0.33	39.9
2. Non-syphilitic positives . . .	86	0.105	13.4
3. Indicative of congenital syphilis . . .	41	0.05	6.4
4. Indicative of acquired syphilis . . .	260	0.32	40.3
Subtotal of adequately studied cases . . .	644	0.78	100.0
5. Inadequately studied cases (unclassified) . . .	134	0.18	
Total . . .	778	0.95	

euphemistically, as "biologic false" or "non-specific" positives. The criteria for all diagnostic categories are discussed in the ensuing paragraphs.

Criteria and Results. Of the 82,070 consecutive specimens, 778 (0.95%) were read as positive or doubtful. A tabulation of the clinical diagnoses arrived at after more detailed study is given in Table 1.

1. *Positive Reactions of Questionable Reliability.* Since "a single positive report cannot be considered valid evidence of syphilis or a biologic false positive reaction,"³ all men whose initial specimens were reported positive and whose repeat tests were all negative, were released from supervision without further follow-up.

The group was comprised of 257 individuals, about 40% of all enrollees adequately studied. Only 31 (roughly 12%) were strong reactors (4+, 3+). The remainder gave weak or partial reactions (2+, doubtful 1+, doubtful ±, negative ±).

2. *Non-syphilitic Positives.* The disposition of sero-positive individuals with no clinical manifestations of syphilis was, in general, that of the Venereal Disease Control Branch of the Army.¹⁷ After 3 months of serologic observations, "men whose serologic reaction reversed to negative are discharged from observations as non-

physical examinations. The patients' reactions were followed bi-weekly by serologic battery and quantitative Kahn tests. When the titer remained constant or manifested a rise the disposition was deferred for at least 3 months before the diagnosis of syphilis was seriously entertained. When the titer fell progressively without treatment, in the absence of any other reason to suspect syphilis, the case was classed as non-syphilitic positive reaction. Cerebrospinal fluid specimens were taken when the blood reactions remained persistently positive. The presence of syphilis in the wife, children, or parents could only be investigated occasionally since few trainees had their families with them. Doubtful instances which could not be observed for a full 3 months, and a few in whom the diagnosis was still uncertain after 3 months, have been classed as "inadequately studied."

Of the total series of enrollees, 86 (0.105%) were diagnosed as non-syphilitic positive reactors. This figure represents 13.4% of the group of positive cases adequately studied.

In this series of subjects, the blood specimens for serology were collected prior to the prophylactic inoculations given new arrivals. Therefore, the influence of such

injections in eliciting positive reactions was in effect only with the small minority of trainees who were enlisting in the Maritime Service freshly after release from the Army or Navy.

3. *Congenital Syphilis.* Syphilis was classified as congenital when the subject gave a history of having positive serology tests or receiving specific treatment during early childhood; or with stigmata such as iritis, keratitis or deformed teeth, which were rarely seen. A corroboratory story was often obtained of the parents or other siblings also receiving treatment. No patient was placed in the congenital class if he gave a history or exhibited any clinical evidences of chancre, secondary rash, or other phenomena of acquired disease.

The diagnosis seemed well established in 41 of the full series (0.05%). This represents 6.4% of the well-studied cases.

4. *Acquired Syphilis.* The nomenclature of the Bureau of Social Hygiene of the City of New York² was utilized for sub-classification:

"A. PRIMARY. Evidence of chancre with or without palpable drainage node. Darkfield positive—serology may be either negative (sero-negative primary) or positive (sero-positive primary).

ment. If duration is unknown, patient less than 25 years of age.

"D. LATE LATENT. (Asymptomatic.) Positive serology and no detectable clinical signs or symptoms. Infection of 4 or more years duration, with or without treatment. If duration is unknown, patient 25 years of age or more.

"E. LATE. Demonstrable clinical signs or symptoms of syphilis of cardiovascular, cerebrospinal, cutaneous, osseous, or other organs and tissues. Confirmatory serology, spinal fluid, or Roentgen examinations."

A total of 260 cases of acquired syphilis were recognized, representing 40.3% of the group of cases adequately studied. The incidence of the various stages is listed in Table 2. More than half of the cases, 149 of the 260, were in the latent stage.

5. *Inadequately Studied.* This category comprises all men who left the training station before a reasonably exact diagnosis could be made. The chief factor responsible for the largeness of this group was the sudden mustering out of trainees which would take place whenever the need for more men by the merchant marine suddenly became pressing. In such urgencies a weakly positive Kahn titer was not

TABLE 2.—AGE GROUP DISTRIBUTION OF ACQUIRED SYPHILIS CASES

Age groups (yrs.)	No. enrollees	Primary (No.)	Secondary (No.)	Early, latent		Late, latent		Late		Total	
				No.	%	No.	%	No.	%	No.	%
16-20 . . .	50,437	0	1	52	0.10	5	0.01	3	0.01	61	0.12
21-25 . . .	11,950	0	1	34	0.28	7	0.06	2	0.02	44	0.40
26-30 . . .	11,930	0	0	37	0.31	23	0.19	3	0.03	63	0.54
31-35 . . .	5,419	0	0	21	0.39	23	0.42	3	0.06	47	0.89
36 and over .	2,334	0	0	5	0.21	35	1.50	3	0.21	45	1.90
Totals . . .	82,070	0	2	149		93		16		260	
% of all 82,070 enrollees		0.0	0.002		0.18		0.11		0.22		0.32

"B. SECONDARY. Evidence of cutaneous, mucous membrane or mucocutaneous lesions with symptoms of generalization, such as adenopathy, early eye involvement or early central nervous system invasion. Wassermann positive.

"C. EARLY LATENT. (Asymptomatic.) Positive serology and no detectable clinical signs or symptoms—infection of less than 4 years duration, with or without treat-

ment. If duration is unknown, patient less than 25 years of age. These men were advised to report to a physician on return from sea. So far as is known, no individual voluntarily disenrolled because of a positive serology finding.

The membership of the unclassified group was found to possess the same age group distribution and the same ratio of weakly to strongly positive reactions,

within the range of normally expected variation, as did the total series of cases adequately studied. It is a reasonable presumption, therefore, that for this unclassified group the diagnostic breakdown was essentially the same as for the series adequately studied. The absolute figures thus far given for the various diagnostic sub-groups may accordingly be augmented by $\frac{134}{644}$ (20.6%) in order to have a more accurate picture of the frequency of the various diagnostic categories within the total population of enrollees.

Comments. *The Age Factor.* Tables 2 and 3 list the incidence of the several conditions discussed in this paper, arranged according to age group class intervals.

The 86 non-syphilitic positive reactors exhibited no significant change from age group to age group, which suggests that, as a general principle, the biochemical influences which give rise to transitorily

tion—early latent syphilis in the men 36 years and older—statistical computation shows that the decline in prevalence lies within the possibility of chance occurrence.

Inasmuch as the Selective Service statistics which have been or will be compiled do not include ages 16 and 17, the years of late adolescence, more detailed data for the age group 16 to 20 years are presented (Table 4). The non-syphilitic positive reactions show no significant change with relation to year of age. Acquired syphilis on the other hand was almost non-existent at 16 years but rose sharply with the succeeding years.

Serologic Patterns of False Positive Reactions. Table 5 is a comparison of reactivity of the 7 member tests of the battery to positive non-syphilitic sera.

In considering this comparison, one must keep in mind that the sero-positivity of

TABLE 3.—COMPARATIVE DISTRIBUTIONS OF NON-SYPHILITIC POSITIVE REACTORS AND OF SYPHILIS CASES IN RELATION TO AGE GROUPS

Age groups (yrs.)	No. enrollees	Non-syphilitic positives		Congenital syphilis		Acquired syphilis		Total syphilis	
		No.	%	No.	%	No.	%	No.	%
16-20	50,437	57	0.11	27	0.05	61	0.12	88	0.17
21-25	11,950	11	0.09	9	0.08	44	0.37	53	0.44
26-30	11,930	11	0.09	3	0.03	63	0.53	66	0.55
31-35	5,419	6	0.11	1	0.02	47	0.87	48	8.90
36 and over	2,334	1	0.04	1	0.04	45	1.93	46	1.97
Totals	82,070	86	0.10	41	0.05	260	0.32	301	0.37

TABLE 4.—COMPARATIVE DISTRIBUTION OF NON-SYPHILITIC POSITIVE REACTORS AND OF CASES OF ACQUIRED SYPHILIS WITHIN THE AGE GROUP 16 TO 20 YEARS

Age (yrs.)	No. enrollees	Acquired syphilis		Non-syphilitic positives	
		No.	%	No.	%
16	12,084	2	0.02	7	0.06
17	16,686	13	0.08	21	0.13
18	14,859	22	0.14	24	0.16
19	3,803	14	0.40	3	0.08
20	3,005	10	0.33	2	0.07

positive responses are active independently of the age factor in adult males.

In congenital syphilis the age groups also show a distribution which appears to be entirely random (Table 3). This to-be-expected relationship vouches for the accuracy of the diagnoses made.

Acquired syphilis, in contrast, increased with ascending age. This relationship held for the sub-groups as well as for the total group. As for the 1 apparent excep-

these individuals was detected originally by the Kahn Standard test, in other specimens collected 2 days to 3 months earlier. Positive Kahn reactions, as expected, had the highest prevalence. Positive Mazzini reactions were the next most numerous. The Kolmer complement fixation test excited less positive reactions than did any of the flocculation tests. A similar lack of "false" reactivity with the Kolmer test had been noted by Reim

and Elsberg¹³ when they surveyed, with a 6-test battery, soldiers whose sera had become transitorily positive in the course of vaccinia, primary atypical pneumonia, and upper respiratory tract infections. These authors commented that in leprosy and malaria, by contrast, the complement fixation tests may demonstrate a higher prevalence of sero-positivity than will the flocculation tests.

proportion of the positive reactions encountered in the initial test may have been positive, not because of extraneous causes, but because their sera at the time of the first test contained reactive substances which had faded away by the time of the second test. If we assume that these statistical influences are mutually neutralizable, the total prevalence of non-syphilitic positives in our series may

TABLE 5.—COMPARATIVE REACTIVITY OF SERA FROM 73 PATIENTS DIAGNOSED AS "NON-SYPHILITIC POSITIVE" TO STANDARD SEROLOGY TESTS

Name of test*	No. sera tested	Negative		Dbtfl. and weakly positive (2+ and less)		Strongly positive (3+, 4+)	
		No.	%	No.	%	No.	%
Kahn Standard	120	29	24	53	44	38	32
Mazzini Flocculation	119	44	37	50	42	25	21
Kline Exclusion	55	24	43	12	40	9	17
Hinton Flocculation	109	68	62	21	19	20	19
Kline Diagnostic	52	33	63	14	27	5	10
Eagle Flocculation	114	80	70	13	10	21	20
Kolmer Complement Fixation	114	96	84	8	7	10	9

* Arranged in order of decreasing reactivity.

All individuals were detected originally by the Kahn Standard test. Every serum listed here was "positive," if only weakly so, to at least one of this battery of tests. Not every test was always performed on each specimen.

Incidence of Non-syphilitic Positives. It is conceivable that some of the individuals placed in this group had latent syphilis, unknown to themselves, or perhaps were deliberately concealing a history of previous antisyphilitic treatment. The number of such syphilitics erroneously termed non-syphilitic positives can only be presumed. The size of the estimate depends upon one's index of suspicion. However, inasmuch as the prevalence of non-syphilitic positive reactions (as diagnosed) was essentially uniform, regardless of age, in contrast with the prevalence of acquired syphilis, which rose concurrently with advance in age, it may be presumed that the number of unrecognized instances of syphilis mistakenly placed in the non-syphilis group was probably not appreciable.

Some of the trainees who gave positive or doubtful reactions on arrival might have been found negative on later study were it not for the stimulating effect of the multiple inoculations (against typhus, smallpox, typhoid and paratyphoid, tetanus, yellow fever) which were given coincidentally with the follow-up serology tests. Conversely, a certain but unknown

be taken as 13.4% of 778, or 104; approximately 1 in 790 for the total population. This rate is somewhat higher, though of the same order of magnitude, as Eagle's "uncorrected" rate of 1 in 1126 college students⁵ and is essentially the same as the prevalence in Stokes' series of 1 in 708 Red Cross donors.¹⁶

Weak vs. Strong Reactions. The strength of the reactivity was related to the occurrence of syphilis as follows: Of 345 weakly positive specimens only 65 (18.8%) were adjudged due to syphilis. Of 299 strongly positive reactions, 236 (78.9%) were adjudged due to syphilis (Table 6; Fig. 1). To generalize from these data, a weakly or partially positive reaction with the Kahn Standard test means that the probabilities are 4 out of 5 that syphilis cannot be demonstrated by careful study. Conversely, a strongly positive reaction means that the probabilities are 4 out of 5 that a syphilitic infection is clearly present.

Prevalence of Syphilis. The prevalence of sero-positive syphilis in this series was approximately 46.7% of 778, or 363; this gives a rate of 4.4 per 1000 and is ap-

proximately one-tenth of that estimated by Vonderlehr and Usilton¹⁸ for the northeastern United States. Their compilation comprised all males between 21 and 35, whereas 60% of the men here surveyed were of age 16 through 20, when syphilis is at its lowest. Moreover, Negroes formed but a small fraction of our series.

It will be recalled that in Stokes' series only 40.5% of the Red Cross donors with positive reacting bloods were finally judged to have syphilis.

Summary. A survey has been made of the incidence and meaning of 783 positive reactions to the Kahn Standard serologic test for syphilis, encountered in a population of 82,070 consecutively examined

TABLE 6.—DISTRIBUTION OF WEAK AND STRONG SEROLOGIC RESPONSES (TO STANDARD KAHN TEST) WITHIN THE DIFFERENT DIAGNOSTIC GROUPS

	Original Kahn test weakly positive (neg. ±, dbt. ±; dbt. 1+, pos. 2+)		Original Kahn test strongly positive (3+, 4+)	
	No.	%	No.	%
Single positives of questionable reliability	226	88	31	12
Non-syphilitic positives	54	63	32	27
Indicative of congenital syphilis	11	27	30	63
Indicative of acquired syphilis	54	21	206	79
Subtotal of adequately studied cases	345	54	299	46
Inadequately studied cases (unclassified)	75	56	59	44
Total	420	54	358	46

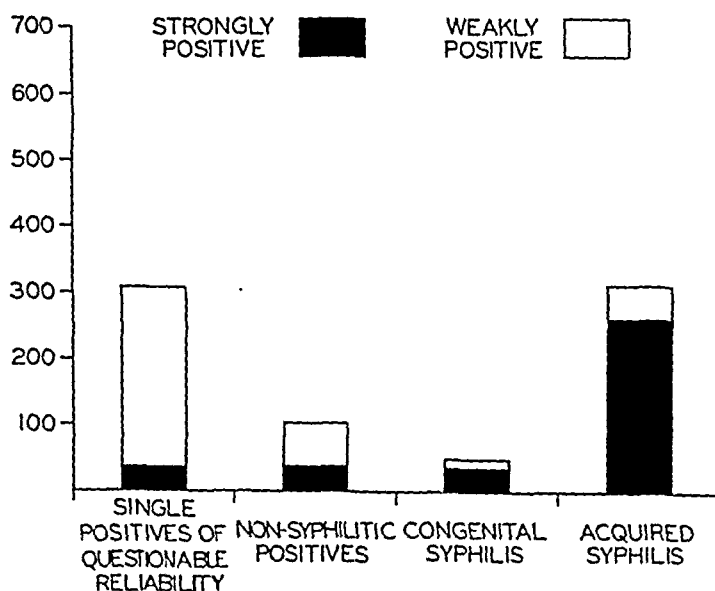


FIG. 1.—Graphic presentation of the distribution of the diagnoses made after clinical study of all positive Kahn reactors encountered among the 82,070 consecutively examined enrollees. "Strongly Positive" refers to those whose first tests were read as 3+ or 4+. "Weakly Positive" comprises all with titers read as 2+ or lower.

Another reason for the difference probably lies in the criteria used for diagnosis. The above authors, and the others cited in the introduction of this paper, based their diagnoses primarily upon the results of serologic tests, whereas in this series the presence or absence of confirmatory findings in history and physical examination were also taken into consideration.

male Maritime enrollees aged 16 to 54 years.

Diagnostic study of the men with positive sera indicated (in round numbers): 40% had single positive reactions of questionable authenticity, 13% had non-syphilitic persistently positive reactions, 7% had congenital syphilis, and 40% had acquired syphilis. The rates for non-

syphilitic positive reactions and for congenital syphilis were essentially constant with all age groups. The rate for acquired syphilis increased progressively with advance in years.

Thus, in one laboratory's experience, the findings of a positive or doubtful response

to a Kahn Standard serologic test led to the diagnosis of syphilis in 47%, or less than half, of a group of healthy American men. Weakly positive reactions proved to mean syphilis in only 20% of the group, whereas strongly positive reactions proved to mean syphilis in 80% of the group.

The majority of patients with positive serology here described were studied and diagnosed by Dr. John J. Kobes, Surg. (R), U. S. P. H. S. The serologic battery was performed under the direction of Dr. J. F. Mahoney, Medical Director, U. S. P. H. S. I am indebted to both of these energetic syphilologists for making their records available, and also to Dr. John Fertig, Professor of Biostatistics, Columbia University, for critical appraisal of the statistical comments in this paper.

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INTESTINAL PARASITES DIAGNOSED AT AN ARMY GENERAL HOSPITAL IN THE SOUTH PACIFIC

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WITH the onset of the war in the Pacific area, an increase in intestinal parasitic infection had been expected by medical practitioners, parasitologists, and other laboratory workers. Over a period of 2 years, a large number of patients, who have had duty in various islands in the South Pacific, have been admitted to a general hospital in that area for varied clinical conditions. During the course of their hospitalization, it has been possible to examine a number of these patients for intestinal parasites, and ultimately to analyze the incidence of both protozoan and helminth infections. Further, as a result of this work, it has been possible to compare our results with surveys carried out by various workers^{2,3,6,10,12,13} among personnel in different locales in the United States, in South America, and in the South Pacific. Mumford and Mohr¹¹ have, in a very excellent tabulation, given the type and the distribution of parasites in the islands of the South Pacific. We believe that our analysis may help in keeping this information up to date.

Material and Methods. Our material is based upon stool studies from soldier patients who have spent up to 3 years in the various islands in the South Pacific area. The patients varied both in duration and degree of exposure and in the islands in which they resided. These patients arrived from other medical installations usually without a previous laboratory diagnosis. Examination for intestinal parasites were carried out on these patients because of (a) clinical findings suggestive of parasitic infection, (b) elevated eosinophil count, (c) fever of unknown etiology, (d) previous diagnosis of an intestinal parasitic infection; in this final group, some had been given previous therapy.

Stool examinations were carried out by direct smears using both saline and D'Antoni's iodine.⁵ It was customary to run a routine iodine stained smear on every stool. Saline smears were made on all fluid stools and at least once for each patient, regardless of the consistency of the stool. In addition to the direct smears, a simplified procedure of the Faust concentration method⁴ was carried out routinely. A suspension of a small particle of a stool made in zinc sulfate (sp. gr. 1.180) was filtered through several layers of gauze into a shell vial. This vial was filled to the top with zinc sulfate. A coverslip was then placed over the top of the vial and permitted to remain in contact with the zinc sulfate for 45 minutes. It was then removed and placed on a slide and examined with the aid of the iodine stain. In addition to the above routine procedures, permanent smears were obtained in cases of doubtful identity. These smears were fixed in Schaudinn's fluid and stained with Heidenhain's hematoxylin. A further technique utilized was a modification of the Baermann procedure¹ for the detection of helminth larvæ. This technique was employed in suspected cases of *Strongyloides*. A large particle of the stool was placed in a small wire meshed basket lined with gauze. This was then placed in a funnel and a volume of water added so that the entire stool specimen was just submerged. After incubation for 12 to 24 hours, 10 ml. was drawn off, centrifuged, and examined for larvæ. In special instances, the swab method of van Hove¹⁴ was used for the detection of *Enterobius vermicularis*. Proctoscopic swabs were obtained at the discretion of the ward officer and sent to the laboratory for examination. Direct saline smears were always made from these specimens and examined immediately for trophozoites.

Results. (a) *Protozoa*. This series involves a total of 4323 stool examinations on

1114 patients, with an average of 3.8 examinations per patient. Table 1 reveals the incidence obtained in this survey. Our results for *Endamæba histolytica* (4.9%) are comparable with those of Boeck and Stiles for the United States and indicate only a slightly higher incidence of amebiasis. This figure is definitely lower than the value of 8.09% obtained by Markell¹⁰ for *E. histolytica*. The results on the incidence of the other protozoans compare favorably with those of Markell. Low incidence for *Giardia* may be explained by the fact that many of these patients have received atabrine, which is an effective therapeutic agent in giardiasis (Love and Taylor⁷). We should like to point out that there is a low incidence for the other intestinal flagellates. It may be possible that these forms, like *Giardia*, are susceptible to atabrine.

bility of the presence of this organism as a contaminant.

During routine microscopic examinations of many specimens of gastric fluid and bile, several cases harboring parasites were discovered. Two were found with *Trichomonas hominis*, 2 with *Enteromonas hominis*, and 1 with *Embadomonas intestinalis*. One biliary drainage was obtained showing tremendous numbers of *Giardia lamblia*.

(b) *Helminths*. The incidence of helminth infections is based on a series of 3415 patients. In this group, a total of 11,358 stool examinations were carried out with an average of 3.3 stool examinations per patient. The data (Table 2) indicate a definitely higher incidence of hookworm infection as compared with the reports from the United States. We obtained an incidence of 13.2% for hookworm as com-

TABLE 1.—INCIDENCE OF INTESTINAL PROTOZOA OF MAN

Author:	Boeck and Stiles	Hopp	Rothman and Laskey	Burrows	Romeu	Markell	Present study
Locale:	U. S. A.	Indiana	Phila.	So. Car.	Brazil	So. Pacific	So. Pacific
No. patients examined:	8029 (%)	771 (%)	306 (%)	2055 (%)	2500 (%)	1371 (%)	1114 (%)
Protozoa							
<i>Endamæba histolytica</i>	4 1	3 1	3 6	1 7	10 4	8 09	4 90
<i>Endamæba coli</i>	19 6	61 9	17 3	16 3	...	13.66	25 10
<i>Endolimax nana</i>	13 2	31 0	12 7	12 4	..	22 50	19 40
<i>Iodamæba bütschlii</i>	5 0	3 8	0 7	0 3	..	3 72	4 10
<i>Dientamæba fragilis</i>	2 3	1 60	..
<i>Giardia lamblia</i>	6 5	1 9	10 0	1 9	10 0	1 96	1 50
<i>Chilomastix mesnili</i>	3 1	8 8	0 7	3 3	..	0 32	0 28
<i>Trichomonas hominis</i>	..	0 1	..	3 3	..	0 40	1 40
<i>Enteromonas hominis</i>	0 3	0 16	1 20
<i>Embadomonas intestinalis</i>	0 35
<i>Isospora hominis</i>	0 37	0 35

The coccidian, *Isospora hominis*, was found in 4 cases of this series. The pathogenicity of this organism is still questionable (Magath⁹). Markell¹⁰ points out that some 200 cases have been reported in man thus far. He found 5 additional cases in his series, of which only 1 showed effects of parasitism, namely dysentery. In the present series 2 patients presented *I. hominis* as a single infection. Of these 2, 1 had a mild diarrhea and enterocolitis. The other case presented no intestinal disturbances. In all instances the oöcysts were found present in the stools on 3 to 5 examinations, thus ruling out the possi-

pared with 3.9% obtained in the survey of Boeck and Stiles. Furthermore, our results are higher than those of Markell's¹⁰ survey from the South Pacific and slightly lower than Romeu's figure of 14.6% for *Necator americanus* in a tropical area of Brazil. In this study hookworm infections are as frequent in soldiers from the northern states as from the southern states. Consequently, we feel that the increased parasitism was acquired in the South Pacific. In 3 instances, adult hookworms were recovered from the stools and the species was determined. In all 3 cases, it was found to be *Ancylostoma duodenale*.

Heterodera radiculicola (*marioni*) is a common plant parasite. According to Craig and Faust,⁴ this nematode has been reported in man, but there is no evidence that it is a true parasite in the human. In the present series, 6 cases were obtained. In all instances, with 1 exception, *H. radiculicola* was associated with multiple parasitic infection. Clinical findings were not associated with the presence of this organism.

the zinc sulfate flotation method on all stools, only 65.8% were positive at the first examination, 88.4% were positive at the third examination and 95.1% were positive at the fifth examination.

The data in Table 4 are self-explanatory and indicates the number of multiple infections.

One instance of intestinal myiasis was obtained. The fly larvæ were recovered from the stools and identified as *Sarco-*

TABLE 2.—INCIDENCE OF INTESTINAL HELMINTHS OF MAN

Author:	Boeck and Stiles	Hopp	Burrows	Romeu	Markell	Present study
Locale:	U. S. A.	Indiana	So. Car.	Brazil	So. Pacific	So. Pacific
No. patients examined:	8029	771	2055	2500	1371	3415
	(%)	(%)	(%)	(%)	(%)	(%)
Helminths						
Hookworm	3 9	...	4 5	14 6	8 46	13 20
<i>Trichuris trichiura</i>	2 1	0 3	0 8	20 5	2 12	2 40
<i>Strongyloides stercoralis</i>	0 1	3 2	0 8	8 6	1 38	1 50
<i>Ascaris lumbricoides</i>	1 1	..	1 4	19.5	0 37	0 67
<i>Enterobius vermicularis</i>	0 8	7 4	0 5	..	0 07	0 41
<i>Hymenolepis nana</i>	0 5	0 1	0 5	...	0 07	0 23
<i>Schistosoma mansonia</i>	4 0	..	0 18
<i>Heterodera radiculicola</i>	0 07
<i>Tænia saginata</i>	0 07	..
<i>Tænia</i> sp.	0 03

TABLE 3.—ANALYSIS OF THE NUMBER OF EXAMINATIONS REQUIRED FOR A POSITIVE LABORATORY DIAGNOSIS OF 165 PATIENTS INFECTED WITH HOOKWORM

No. examinations	Increase in No. new positives with each examination	Total No. positive	Positive diagnosed with successive stool examinations (%)
1	126	126	76 3
2	22	148	89 6
3	9	157	95 1
4	5	162	98 1
5	2	164	99 3
6	1	165	100 0

A single case of tæniasis was discovered, but further identification was impossible as no segments were recovered. In 1 case many larvæ of *Strongyloides stercoralis* were obtained during biliary drainage.

A question that interested us was to determine the number of examinations required before a negative diagnosis may be accepted. For this study, we decided to use the hookworm data (Table 3). In a series of 165 patients, 76.3% were positive with only 1 examination, while 3 examinations gave 95.1% positive. After 5 examinations, 99.3% were positive. In a similar survey on an earlier group of men, during which time we were unable to use

phaga sp., which has already been described in the literature as one of the etiologic agents of intestinal myiasis (Lyon and Mizelle⁸).

Summary. 1. A survey was made for intestinal parasites in soldier patients (15,681 examinations) from an Army General Hospital located in the South Pacific area.

2. The incidence of protozoan infection in 1114 patients is 4.9% with *Endamaba histolytica*; 25.1% with *Endamaba coli*; 19.4% with *Endolimaz nana*; 4.1% with *Iodamaba bütschlii*; 1.5% with *Giardia lamblia*; 1.4% with *Trichomonas hominis*; 1.2% with *Enteromonas hominis*; 0.28%

TABLE 4.—MULTIPLE PARASITIC INFECTIONS

Double Infections	
<i>E. coli</i> and <i>E. nana</i>	34
<i>E. coli</i> and hookworm	21
<i>E. coli</i> and <i>E. histolytica</i>	9
<i>E. coli</i> and <i>I. bütschlii</i>	7
<i>E. coli</i> and <i>T. trichiura</i>	5
<i>E. coli</i> and <i>S. stercoralis</i>	2
<i>E. coli</i> and <i>H. radiculicola</i>	1
<i>E. coli</i> and <i>C. mesnili</i>	1
<i>E. coli</i> and <i>G. lamblia</i>	1
<i>E. nana</i> and hookworm	10
<i>E. nana</i> and <i>E. histolytica</i>	6
<i>E. nana</i> and <i>T. trichiura</i>	5
<i>E. nana</i> and <i>I. bütschlii</i>	4
<i>E. nana</i> and <i>T. hominis</i>	2
<i>E. nana</i> and <i>E. hominis</i>	2
<i>E. nana</i> and <i>G. lamblia</i>	1
<i>E. nana</i> and <i>S. stercoralis</i>	1
<i>I. bütschlii</i> and hookworm	4
<i>I. bütschlii</i> and <i>E. histolytica</i>	3
<i>I. bütschlii</i> and <i>G. lamblia</i>	1
<i>I. bütschlii</i> and <i>E. hominis</i>	1
<i>E. histolytica</i> and hookworm	2
<i>E. histolytica</i> and <i>C. mesnili</i>	1
<i>E. histolytica</i> and <i>E. hominis</i>	1
<i>E. histolytica</i> and <i>G. lamblia</i>	1
<i>G. lamblia</i> and hookworm	1
<i>T. hominis</i> and hookworm	3
<i>T. hominis</i> and <i>E. vermicularis</i>	1
<i>T. trichiura</i> and hookworm	3
<i>T. trichiura</i> and <i>S. stercoralis</i>	2
<i>T. trichiura</i> and <i>H. radiculicola</i>	1
<i>T. trichiura</i> and <i>A. lumbricoides</i>	1
Hookworm and <i>S. stercoralis</i>	3
Hookworm and <i>H. radiculicola</i>	1
Triple Infections	
<i>E. coli</i> , <i>E. nana</i> and hookworm	7
<i>E. coli</i> , <i>E. nana</i> and <i>E. histolytica</i>	6
<i>E. coli</i> , <i>E. nana</i> and <i>I. bütschlii</i>	5
<i>E. coli</i> , <i>E. nana</i> and <i>C. mesnili</i>	1
<i>E. coli</i> , <i>E. nana</i> and <i>T. hominis</i>	1
<i>E. coli</i> , <i>E. nana</i> and <i>T. trichiura</i>	1
<i>E. coli</i> , <i>I. bütschlii</i> and <i>E. histolytica</i>	2
<i>E. coli</i> , <i>S. stercoralis</i> and hookworm	2
<i>E. coli</i> , <i>E. histolytica</i> and hookworm	2
<i>E. coli</i> , <i>E. histolytica</i> and <i>C. mesnili</i>	1
<i>E. coli</i> , <i>E. histolytica</i> and <i>E. hominis</i>	1
<i>E. coli</i> , <i>I. bütschlii</i> and <i>S. stercoralis</i>	1
<i>E. coli</i> , <i>E. histolytica</i> and <i>H. radiculicola</i>	1
<i>E. coli</i> , <i>T. trichiura</i> and hookworm	1
<i>E. coli</i> , <i>I. hominis</i> and <i>T. trichiura</i>	1
<i>E. coli</i> , <i>H. nana</i> and hookworm	1
<i>E. coli</i> , <i>H. radiculicola</i> and hookworm	1
<i>E. nana</i> , <i>T. trichiura</i> and hookworm	3
<i>E. nana</i> , <i>S. stercoralis</i> and hookworm	3
<i>E. nana</i> , <i>T. hominis</i> and hookworm	1
<i>E. nana</i> , <i>T. hominis</i> and <i>G. lamblia</i>	1
<i>S. stercoralis</i> , <i>T. trichiura</i> and hookworm	1
Quadruple Infections	
<i>E. coli</i> , <i>E. histolytica</i> , <i>S. stercoralis</i> and hookworm	2
<i>E. coli</i> , <i>E. histolytica</i> , <i>E. nana</i> and hookworm	1
<i>E. coli</i> , <i>E. histolytica</i> , <i>E. nana</i> and <i>I. bütschlii</i>	1
<i>E. coli</i> , <i>E. histolytica</i> , <i>S. stercoralis</i> and <i>T. trichiura</i>	1
<i>E. coli</i> , <i>E. nana</i> , <i>G. lamblia</i> and <i>H. radiculicola</i>	1
<i>E. coli</i> , <i>I. bütschlii</i> , <i>E. hominis</i> and <i>T. trichiura</i>	1
<i>E. coli</i> , <i>S. stercoralis</i> , <i>E. intestinalis</i> and hookworm	1
<i>E. nana</i> , <i>I. bütschlii</i> , <i>I. hominis</i> and hookworm	1

with *Chilomastix mesnili*; 0.35 % with *Iso-spora hominis*; and 0.35 % with *Embad-omonas intestinalis*.

3. The incidence of helminth infection in 3,415 patients is 13.2 % with hookworm; 2.4 % with *Trichuris trichiura*; 1.5 % with *Strongyloides stercoralis*; 0.67 % with *Ascaris lumbricoides*; 0.41 % with *Enterobius vermicularis*; 0.25 % with *Hymenolepis nana*; 0.18 % with *Heterodera radiculicola*; and 0.03 % with *Tænia* sp.

4. The incidence of hookworm in the present survey is higher than those recorded on the surveys from the United States.

5. Recovery of adult hookworm in 3

cases showed the species to be *Ancylostoma duodenale*.

6. Analysis of the data on 165 cases of hookworm showed that 76.3 % were positive after 1 examination; 95.1 % were positive after 3 examinations and 99.3 % after 5 examinations.

7. It is suggested that atabrine prophylaxis commonly used in this area explains not only the low incidence of *Giardia*, but also the low incidence of the other intestinal flagellates.

8. Four new cases of *Isospora hominis* are noted.

9. One new case of intestinal myiasis, due to *Sarcophaga* sp. is reported.

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EFFECT OF GRADED DOSES OF THYROXIN ON EXPERIMENTAL GOITERS, INDUCED BY PROMIZOLE*

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THE thyroid stimulating effect of the more recently identified iodine resistant goitrogens is thought to be due to the inability of the thyroid gland to synthesize thyroxine at levels adequate to maintain normal metabolism.¹ This reduction of available thyroid hormone presumably permits an increase of the elaboration of thyrotropic hormone by the anterior lobe of the pituitary, resulting in the production of the goiters. Thyroxin, when provided parenterally during the time the goitrogen is given, will completely prevent the thyroid changes. Three to 10 μ g. daily prevented thyroid changes in animals fed a Brassica seed diet.¹³ Goiters produced by sulfaguanidine¹⁰ were entirely prevented by giving thyroxin at a level of 1 μ g. for each 10 gm. of body weight. Thyroid hyperplasia was abolished by the addition of 1% of thyroid powder to the diet of animals which were given sulfaguanidine² and was effectively prevented by giving 5 μ g. of thyroxin daily to animals which were given thiouracil.⁴ Between 2 and 3 μ g. of thyroxin daily prevented thyroid enlargement in both male and female chicks receiving 0.1% of thiouracil in their diet for 14 days.¹² Changes similar to those seen in cretinism, which were induced in young rats given thiouracil, were prevented by administration of thyroxin.⁹ Metabolic rates were returned to normal in animals receiving thiouracil by the daily injection of 4.75 μ g. of d,1-thyroxin, while 4.8 μ g. were required to return the weights of the thyroid glands to normal levels.¹⁴

Promizole, a sulfone shown to exert a favorable influence on experimental tuberculosis, is likewise goitrogenic.^{5,8} The thyroid hyperplasia observed in animals given promizole could not be prevented by giving large amounts of iodine but was completely controlled by giving thyroxin. Furthermore, the hyperplasia of the thyroid gland did not occur in animals, previously hypophysectomized, when given the goitrogen.⁷ Data suggest the conclusion that promizole, like the thiourea derivatives, exerts its influence by inhibiting the synthesis of thyroxine resulting in an increased elaboration of thyrotropic hormone by the anterior lobe of the pituitary.

This report covers the results of a study undertaken to determine the amount of thyroxin necessary to prevent the changes of the thyroid gland which promizole will induce in animals.

Method. Forty young male rats having an average weight of 65 gm. were selected for this study. They were arranged into 8 groups of 5 rats each. Five were fed the standard ration (Friskies) without the goitrogen, constituting Control Group 1. Five ate the ration to which promizole had been added at a level of 0.5% and constituted Control Group 2. The animals of the 6 remaining groups ate the diet containing promizole but were given, intraperitoneally, solutions of d,1-thyroxin† containing the following daily amounts: 1, 2, 4, 6, 8 and 10 μ g. respectively. The experiment continued for 28 days.

At the end of this test period the oxygen consumption of each animal was recorded

* Promizole (4,2'-diaminophenyl-5'-thiazolyl sulfone) was made available for this study through the courtesy of Drs. E. A. Sharp and L. A. Sweet of Parke, Davis & Co.

† Made available for our use through the courtesy of Dr. E. C. Kendall, Mayo Clinic, Rochester, Minnesota.

and the average metabolic rate was computed in calories per square meter per hour, using the method previously described.³ Samples of blood were taken from the heart of each animal and, using standardized pipets, the total red and white cell counts were determined. Using the Van Allen hematocrit tubes, red cell volumes in cubic microns were determined and the grams of hemoglobin per 100 cc. of blood were read on the Cenco-Sheard-Sanford hemoglobinometer. Differential distributions of the leukocytes were determined from smears appropriately stained.

The thyroid and pituitary glands were removed from etherized animals, weighed on a precision balance to 0.1 mg. and fixed in formalin. Thyroids were sectioned at 3 microns and stained with hematoxylin and eosin. Using an oil immersion lens ($\times 90$) and a $6\times$ ocular with an echelon micrometer scale, the heights in microns of 200 thyroid acinar cells were measured for each animal in Control Group 1 and 100 acinar cells were measured for Control Group 2 and for each test animal which had received thyroxine. The means with their probable errors were computed.

Results. The daily administration of thyroxine to the promizole fed rats did not counteract the toxic effects of the drug, so as to induce increased or normal rates of growth (Table 1).

The data on the average oxygen consumption of the animals are condensed into Table 2 and graphically shown in Figure 1. Promizole (Control Group 2) decreased the metabolic rate during the 28 days, 19.7% below that of the normal Control Group 1. Two μ g. of thyroxine daily kept the metabolic rate 10.4% higher than that of Control Group 2 but 11.3% below the normal Control Group 1. Four μ g. of thyroxine daily maintained an oxygen consumption equal to that observed in animals of Control Group 1. Six μ g. daily raised the rate significantly, but only slightly so; while 10 μ g. per day induced a real increase of the amounts of oxygen consumed (Table 2).

Daily administration of thyroxine to rats eating the promizole ration, lessened the extent of hyperplasia of the thyroid,

TABLE 1.—THYROID AND PITUITARY GLAND WEIGHTS OF ALL ANIMALS AT THE END OF THE EXPERIMENT

Group*	Attained body wt. (gm.)	Thyroxine daily (μ g.)	Thyroid wt. (mg.)		Pituitary wt. (mg.)	
			Absolute	Per 100 gm. body weight	Absolute	Per 100 gm. body weight
Control 1	145.6	0	9.2 \pm 0.4†	6.3 \pm 0.3	5.1 \pm 0.3	3.5 \pm 0.1
Control 2	91.0	0	30.5 \pm 2.0	33.5 \pm 1.0	4.8 \pm 0.3	5.2 \pm 0.3
Test 1	97.6	1	17.5 \pm 2.2	17.7 \pm 1.9	3.4 \pm 0.2	3.5 \pm 0.1
Test 2	76.8	2	9.1 \pm 0.4	11.8 \pm 0.3	2.4 \pm 0.1	3.1 \pm 0.1
Test 3	80.1	4	7.2 \pm 0.2	9.0 \pm 0.4	2.6 \pm 0.1	3.2 \pm 0.1
Test 4	83.0	6	8.7 \pm 0.5	10.1 \pm 0.4	3.2 \pm 0.2	3.8 \pm 0.2
Test 5	89.2	8	6.9 \pm 0.3	7.6 \pm 0.1	3.0 \pm 0.3	3.4 \pm 0.1
Test 6	86.0	10	7.7 \pm 0.3	8.9 \pm 0.3	2.8 \pm 0.2	3.2 \pm 0.3

* Each group contained 5 rats.

† Probable error of the mean.

TABLE 2.—MEAN OXYGEN CONSUMPTION

Group	Thyroxine daily (μ g.)	Mean oxygen consumption (calories per sq. m. per hr.)	Difference	
			Calories	%
Control 1	0	44.2 \pm 0.7*		
Control 2	0	35.5 \pm 1.3	-8.7 \pm 1.5†	-19.7
Test 2	2	39.2 \pm 1.3	3.7 \pm 1.8‡	10.4
Test 3	4	46.1 \pm 1.8	10.6 \pm 2.2‡	29.9
Test 4	6	56.5 \pm 2.5	21.0 \pm 2.8‡	50.2
Test 5	8	50.8 \pm 0.6	15.3 \pm 1.4‡	43.1
Test 6	10	58.9 \pm 0.7	23.4 \pm 1.5‡	65.9

* Probable error of the mean.

† Control Group 2 - Control Group 1.

‡ Respective test group - Control Group 2.

as had been anticipated (Table 1). Promizole in the diet (Control Group 2) produced goiters with a relative weight more than 5 times, and an absolute weight more than 3 times that of the thyroids in the normal Control Group 1. One $\mu\text{g.}$ of thyroxin daily restricted the thyroid hyperplasia to about a half, and 2 $\mu\text{g.}$

animals (Table 1) were of interest in that the administration of the thyroid hormone did result in maintaining smaller pituitaries in promizole fed rats. Even in small amounts thyroxin appeared to inhibit pituitary changes so that both absolute and relative weights were significantly less than the average pituitary weights in

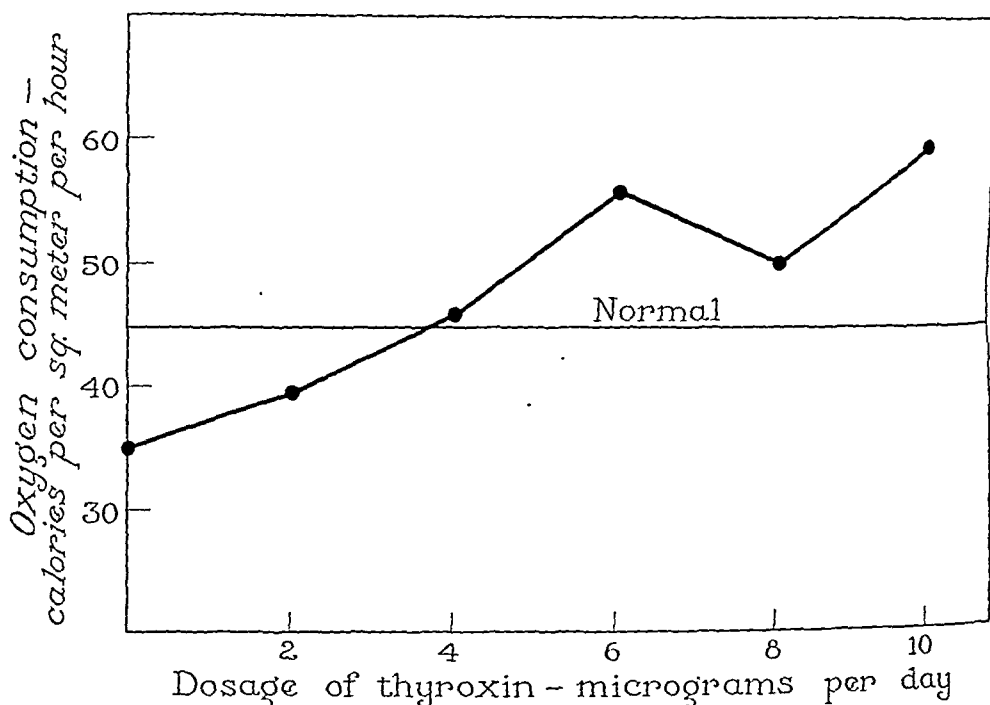


Fig. 1.—Changes in calories per square meter per hour recorded at the end of the experiment. Normal data were obtained from Control Group 1.

to about a third of that observed in animals not given supplementary hormone. Four $\mu\text{g.}$ of thyroxin daily reduced the weights of the thyroids considerably below the average weight in the normal control animals. Larger amounts did not further reduce the average thyroid weights. Mere shreds or residues of colloid remained in acini of thyroids which had been subjected to the influence of promizole (Fig. 2). Thyroxin counteracted this effect. One $\mu\text{g.}$ per day greatly lessened these changes in the colloid patterns and 2 $\mu\text{g.}$ per day completely nullified them (Fig. 3).

Both the absolute and the relative weights of the pituitary glands of these

animals which received the goitrogen but were not given the hormone.

The measurements of the thyroid acinar cell heights have been condensed into Table 3. The percentage distributions of these data within 4 of the 8 groups are shown in Figure 4. According to Figure 5, a daily amount of the hormone equal to about 1.75 $\mu\text{g.}$ was sufficient to prevent any increase in size of the cells comprising the thyroid epithelium.

The data assembled from heart samples of blood are condensed into Tables 4 and 5. Promizole induced a slight anemia, with lowered red cell counts and increased red cell volumes, but the hemoglobin levels were not significantly reduced. This

standard ration provides very much more protection to animals receiving promizole than do purified rations fortified by the better known vitamin B fractions. The average total leukocyte count, however, was greatly reduced from control levels in all animals given promizole.

Although there were trends to suggest

that thyroxin may have had some effect in counteracting the destructive influence of promizole on the red cells, yet the data are not too impressive. Certainly the hemoglobin levels were not significantly altered by administration of thyroxin and the leukopenia observed to accompany the feeding of promizole in this experiment

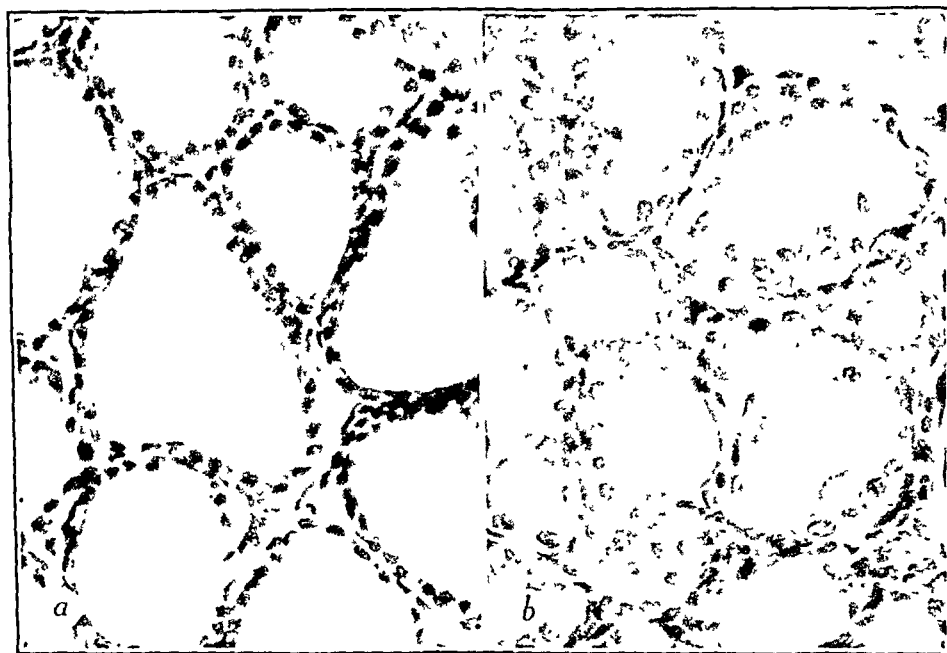


FIG. 2.—Histologic appearance of thyroid glands. *a*, Control Group 1, which did not receive promizole. *b*, Control Group 2, which received promizole in the diet at a level of 0.5% in ration.

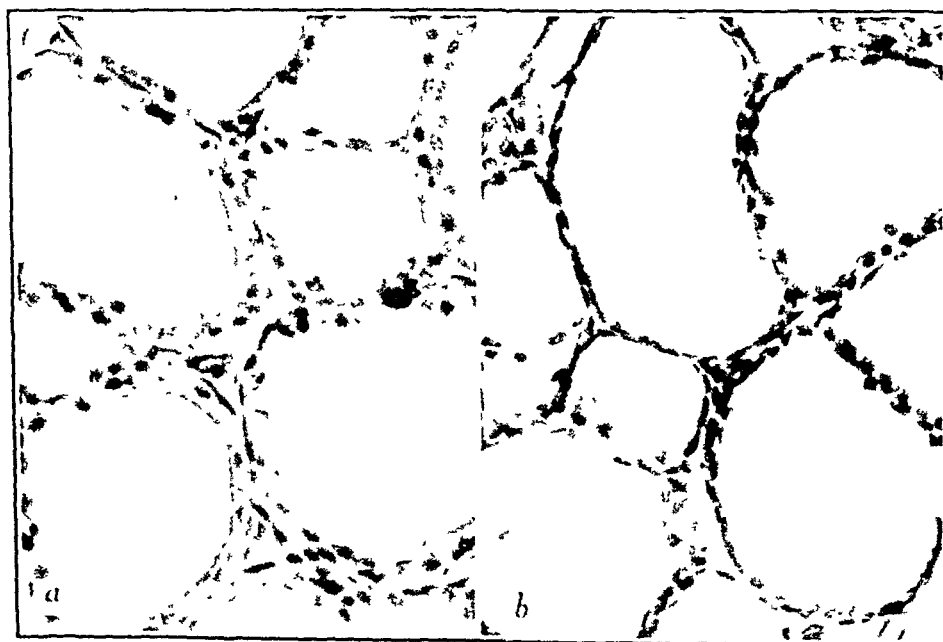


FIG. 3.—Histologic appearance of thyroid glands. *a*, Test Group 1, which received promizole and 1 µg. of thyroxin daily. *b*, Test Group 2, which received promizole and 2 µg. of thyroxin daily.

was not corrected by giving thyroxin (Table 4). But a study of the differential distribution data showed a reaction of the neutrophilic granulocytes to thyroxin that would seem to warrant further study (Table 5). Significant elevations were observed to occur in the percentages of granulocytes in the blood of all animals which received 2, 6 or 8 $\mu\text{g.}$ of the hormone daily. Among animals receiving 10 $\mu\text{g.}$ daily, the scatter of the data rendered a satisfactory deduction impossible. Significant changes in the monocytes or the eosinophilic or basophilic granulocytes did not occur in the hormone treated animals.

Comment. The toxic reactions sustained by young animals given promizole, particularly anorexia and failure to grow normally, were not prevented by giving

thyroxin. Thiouracil, given subcutaneously,⁹ inhibited the growth of young rats but when thyroxin was given the normal growth rates were restored. Since thyroxin was without effect on the growth of our promizole fed animals, retardation of development was obviously not due to a deficiency of thyroid hormone.

The decline of the rate of oxygen consumption, however, was the result of a thyroid hormone deficiency, for the administration of the hormone at the level of 2 $\mu\text{g.}$ daily exerted some effect on the amount of oxygen consumed. Slightly less than 4 $\mu\text{g.}$ daily was adequate to prevent any decline of the metabolic rate from the level of Control Group 1. These data on the rapid decline of oxygen consumption are in keeping with those observed when

TABLE 3.—THYROID ACINAR CELL HEIGHTS

Group	Diet	Thyroxin daily ($\mu\text{g.}$)	Thyroid acinar cell height (μ)
Control 1 . . .	Standard	0	4 61 \pm 0 01*
Control 2 . . .	0 5% promizole	0	11.90 \pm 0 10
Test 1	0 5% promizole	1	8 00 \pm 0 10
Test 2	0 5% promizole	2	3 10 \pm 0 02
Test 3	0 5% promizole	4	2 70 \pm 0 02
Test 4	0 5% promizole	6	2 70 \pm 0 02
Test 5	0 5% promizole	8	2 80 \pm 0 03
Test 6	0 5% promizole	10	2 70 \pm 0 03

* Probable error of the mean.

TABLE 4.—BLOOD VALUES OBTAINED FROM SAMPLES OF HEART BLOOD AT THE END OF THE EXPERIMENT

Group	Thyroxin daily ($\mu\text{g.}$)	Erythrocytes		Hemoglobin (gm. per 100 cc. of blood)	Leukocytes (thous. per c.mm.)
		Mill. per c.mm.)	Volumes (c. μ)		
Control 1	0	7 8 \pm 0 1*	49 9 \pm 1 2	14 0 \pm 0 3	15 3 \pm 0 7
Control 2	0	6.7 \pm 0 3	60.1 \pm 0 1	13 1 \pm 0 5	5 9 \pm 0 4
Test 1	1	7 4 \pm 0 2	59 9 \pm 1 3	14 5 \pm 0 5	7 5 \pm 0 8
Test 2	2	7 3 \pm 0 2	55 0 \pm 1 1	13 3 \pm 0 2	5 4 \pm 0 6
Test 3	4	7 4 \pm 0 2	51 0 \pm 1.1	11 9 \pm 0.5	3 7 \pm 0 3
Test 4	6	6 9 \pm 0 2	57 4 \pm 2 4	11 9 \pm 0 6	7.2 \pm 0 8
Test 5	8	7 9 \pm 0 2	48.9 \pm 1 3	12 6 \pm 0.5	5 5 \pm 1.9
Test 6	10	7.5 \pm 0 2	54.2 \pm 0.7	13 8 \pm 0 2	6 4 \pm 0.6

* Probable error of the mean.

TABLE 5.—DIFFERENTIAL DISTRIBUTION OF LEUKOCYTES AT THE END OF THE EXPERIMENT

Group	Thyroxin daily ($\mu\text{g.}$)	Granulocytes				
		Lymphocytes	Monocytes	Neutrophilic	Eosinophilic	Basophilic
Control 1	0	85 7 \pm 1.3*	2.1	11.7 \pm 0 4	0.3	0 2
Control 2	0	82.0 \pm 1 4	1.5	14 2 \pm 1.4	1 8	0 5
Test 1	1	84 2 \pm 1 4	0 9	13.2 \pm 1 0	1.2	0 5
Test 2†	2	77 0 \pm 3 5	0 8	21 2 \pm 1.9	0.6	0 4
Test 4	6	64 0 \pm 5.2	2 1	30 6 \pm 2.5	0.7	2 6
Test 5	8	63 0 \pm 1 4	1.5	35 0 \pm 2 4	0 5	0
Test 6	10	74 0 \pm 3.1	1.0	23 5 \pm 10 6	1 0	0 5

* Probable error of the mean.

† Data on Test Group 3 not obtained.

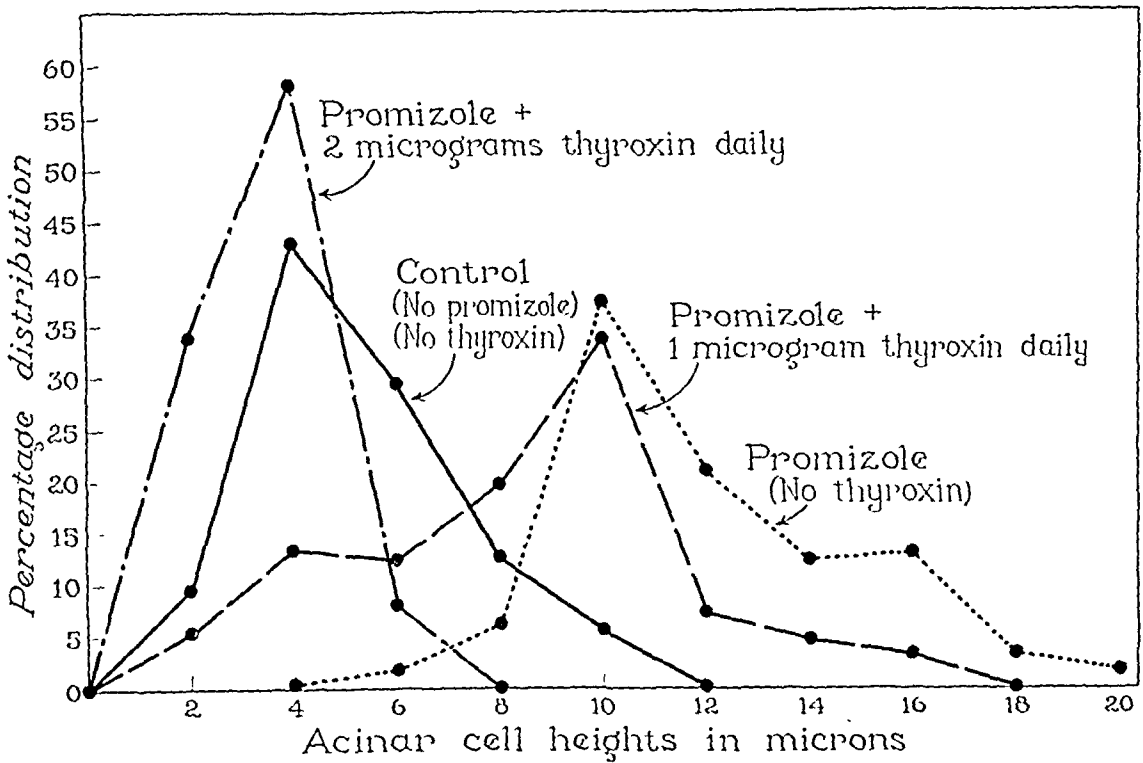


FIG. 4.—Percentage distributions of the heights of the thyroid acinar cells in 4 groups of animals: Control Groups 1 and 2; Test Groups 1 and 2.

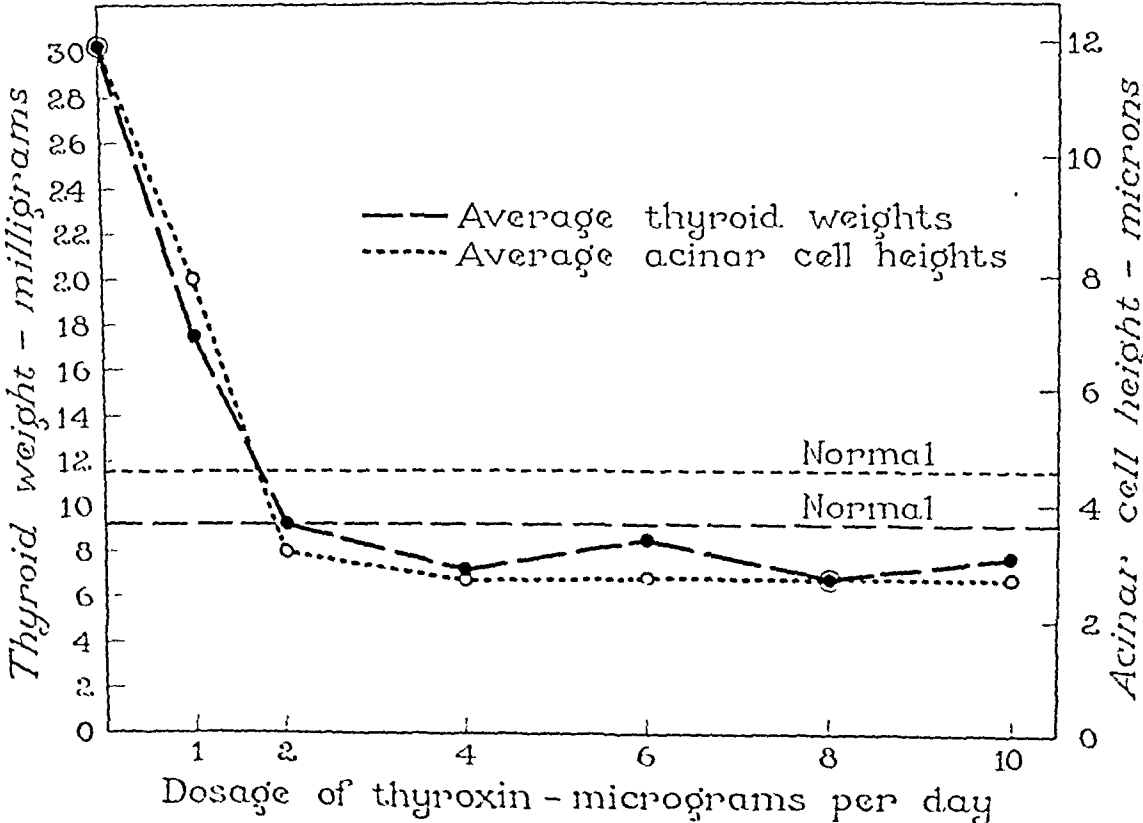


FIG. 5.—Changes in the weights of the thyroid glands and the heights of the thyroid acinar cells in animals which received promizole and in those which received promizole together with graded doses of thyroxin daily. Normal data were obtained from Control Group 1.

sulfaguanidine was given.¹¹ Reineke, Mixer and Turner¹⁴ lowered the metabolic rate 23.7% when thiouracil was provided in the drinking water at a level of 0.1% for 14 days. These rates were returned to normal by the daily injection of 4.75 μ g. of d,l-thyroxin.

The protection afforded by thyroxin to the colloid bodies of animals given promizole is of interest. Promizole, like other goitrogens, produces changes in the metabolism of the thyroid gland resulting in the atrophy or destruction of its colloid reserves. Whereas 1 μ g. of thyroid hormone appeared to provide some protection to the colloid bodies, 2 μ g. provided complete protection. The homogeneous appearance of the colloid was retained; its staining qualities were preserved. The reasons for the retention of normal colloid in thyroid glands of animals receiving promizole when provided adequate blood thyroxin levels are not clear. Since the basal metabolism of such animals was increased when the hormone was given, the thyroid cell, in the presence of an adequate or even increased level of blood thyroxin, may retain its selective function for metabolizing iodine and synthesizing thyroxin. There is need, it would seem, for a study of the capacity of the hyperplastic thyroid to take up radio-iodine in the presence of normal or increased blood levels of thyroxin.

We learned that the blood levels of promizole were progressively lower in animals into which thyroid hormone was injected at the levels which ranged from 1 to 8 μ g. The average concentrations of promizole in the blood stream of the Control Group 2 animals and in the blood of those receiving 1 μ g. of thyroxin daily were statistically alike, ranging from 6.6 to 15 mg. per 100 cc. of blood (average 9.2 mg. per 100 cc.). The average concentrations of promizole in the blood of the remaining groups of animals were as follows: 2 μ g. of thyroxin, 8.2 mg. per 100 cc.; 4 μ g., 7.5 mg. per 100 cc.; 6 μ g., 5.8 mg. per 100 cc.; 8 μ g., 3.7 mg. per 100 cc.; and 10 μ g., 6.3 mg. per 100 cc. Increased rates of metabolism induced by the added

daily amounts of thyroxin may have facilitated the more rapid destruction or elimination of the drug and may serve to explain the absence of the colloid damage.

A more probable explanation for the retention of normal colloid bodies in rats treated with thyroxin and fed promizole is that in the presence of an adequate blood thyroxin level no demands were made on the colloid for thyroid hormone. The colloid bodies thus remained unexhausted and their usual thyroxin reserve remained intact. It is evident that 1 μ g. daily was insufficient to meet the body requirements, so that some demands were made on these colloid reserves. The inability of the thyroid cells to replace them resulted in the atrophic colloid patterns. When 2 μ g. were given daily, the bodily requirements were met and there were no demands made on the thyroid for thyroid hormone, so that normal colloid patterns persisted. The conclusion may well be drawn that the requirement of thyroxin by rats of this age, as used in this experiment, was about 2 μ g. per day.

In our experience with animals to which promizole was fed or into which it was injected, real increases of the weights of the pituitary glands occurred during the production of the goiter. This fact may be correlated with an increased elaboration of the thyroid stimulating hormone. Observations elsewhere have contributed to the conclusion that increases of the relative numbers of basophilic cells did occur in the pituitaries of animals made goitrous by sulfaguanidine.¹⁰ On the other hand, changes of the microscopic structure of pituitaries of animals made goitrous by thiouracil were not observed.¹⁵ Pituitaries of animals made goitrous by a rapeseed diet, however, were reported to contain an increased number of basophilic cells with degranulation of the acidophilic cells.⁵

Whether or not the increased relative weights of the pituitaries of our animals made goitrous by promizole were related to changes of the content or the output of thyroid stimulating hormone, such increased weights did not occur when thyroxin was administered. Even 1 μ g. of thyroxin

per day maintained relative weights and larger amounts of the hormone actually reduced the absolute weights of the pituitary glands. The decline of pituitary gland weights was probably dependent on the size and the extent of hyperplasia in the developing goiter. When normal thyroid gland weights and normal histologic patterns were maintained by the injection of 2 μ g. of thyroid hormone daily, the weights of the pituitary glands were likewise reduced. Accordingly, 2 μ g. of thyroxin per day, or perhaps an amount slightly less, will maintain in rats eating a standard diet containing 0.5% of promizole for 28 days, a normal thyroid gland, with normal or subnormal acinar cell heights, and normal appearing colloid bodies, together with pituitary glands of normal or subnormal weights.

Conclusions. 1. The administration of d,1-thyroxin, in amounts ranging from 1 to 10 μ g. daily, to young rats during a period of 28 days, while they were fed a diet containing the goitrogen, promizole, at a level of 0.5%, did not inhibit the usual untoward effects which promizole normally exerts on the growth, appearance and appetite of immature animals.

2. As hitherto shown, promizole depressed thyroid activity. In this experiment the average metabolic rate of animals given promizole in their diet for 28 days was lowered 19.7% from that recorded for the normal control group. Two μ g. of d,1-thyroxin daily increased the average rate 10.4% above that of the control group which received promizole alone, while 4 μ g. maintained an average oxygen consump-

tion equal to that recorded for the animals which did not receive either thyroxin or promizole.

3. Promizole exerts a thyroid-stimulating influence through the pituitary gland, which, in this experiment, resulted in the development of goiters weighing more than 3 times the average weight of the thyroid gland in control animals. The administration of 1 μ g. of d,1-thyroxin daily resulted in the development of goiters which were but slightly less than twice the normal size, while 2 μ g. daily resulted in maintaining thyroid glands of normal weights.

4. The heights of the thyroid acinar cells were maintained at normal or less than normal levels by giving 2 μ g. of d,1-thyroxin daily. Without any supplemental thyroxin, the average acinar cell height was more than twice that of the normal gland, while 1 μ g. of thyroxin was adequate to maintain an average cell height considerably less than twice that of the normal thyroid gland.

5. The changes observed in the colloid of thyroid acini which were induced by promizole were partially prevented by 1 μ g. of thyroxin per day and were completely nullified by giving 2 μ g. daily.

6. Promizole appears to exert its goiter stimulating effect by preventing the synthesis of thyroxin by the thyroid cell, thereby permitting an increased elaboration of thyroid stimulating hormone by the anterior lobe. By the administration of small amounts of thyroxin daily during the time the goitrogen was provided, thereby maintaining adequate blood levels of thyroxin, normal thyroid glands were maintained.

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NOTE ON HIPPURIC ACID SYNTHESIS IN SENILITY

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IN a previous communication (Stern, Hinds and Askonas⁵), it was demonstrated that hippuric acid synthesis is impaired in higher age. The original purpose of that investigation was to determine the detoxifying capacity of the aging organism, particularly during psychoses of the higher age group. There is a striking similarity between certain senile psychotic episodes and delirious psychoses produced by exogenous toxins during other periods of life. On the basis of this clinical fact, it had been assumed that the capacity to detoxify certain metabolites is reduced in old age.

Only hippuric acid synthesis was studied, because it is the only test which has been sufficiently evaluated quantitatively for clinical purposes. This test was found to be markedly impaired in the group of patients examined. Of patients of 60 years or more, 80% were shown to excrete less than 0.85 gm.; the 5 cases diagnosed as senile dementia ranged from 0.18 to 0.7 gm. This impairment was very marked compared with observations on mentally and physically normal old people (Rafsky and Newman³).

However, the test is an index of 2 functions: the conjugating power of the liver and the capacity to furnish glycine (by synthesis or by mobilization). Therefore an attempt was made to determine as to which one of these factors was impaired in the aged. This was done by the administration of glycine during the test. A similar procedure has been carried out before in patients with liver diseases (Probstein and Londe¹) by the oral sodium benzoate test.

Subjects. Twelve patients with senile or pre-senile conditions ranging from the age of 58 to 92 were examined. As controls 6 healthy adults were used and 4 adults of faulty dietary habits. One of the latter was a woman of 56 who suffered from a peculiar anxiety state with anorexia. NPN, blood cholesterol, blood sugar and, in the senile cases, the albumen/globulin ratio were determined in order to exclude cases with gross metabolic disorders.

Method. A test was carried out on 3 consecutive days. On the 1st and 3rd day Quick's intravenous hippuric acid test was carried out in the usual manner, with 1.77 gm. of sodium benzoate. On the 2nd day glycine was added in the following way.

The subject was given 1 gm. of glycine as a 10% watery solution $\frac{1}{2}$ hour before the injection of sodium benzoate. One gm. of glycine was given orally at the time of the injection; $\frac{1}{2}$ hour after this another gram of glycine was administered. Before the injection and 1 hour following it the urine was collected. In the old patients this was done under the supervision of physician and nurse, and in some cases by catheterization. Each subject was given 2 tumblers of water before the injection to assure a sufficient output of urine. Hippuric acid was determined according to Quick.²

Results. The results are presented in 2 graphs and in Table 1.

The 6 normal control cases show that the hippuric acid values on the 1st and 3rd day correspond to the normal cases given by Quick and do not differ more from one another than 0.2 gm. An additional oral administration of glycine on the 2nd day increased the hippuric acid output by 0.14 to 0.28 gm. in all but 1 case.

Ten out of 12 senile cases showed a low hippuric acid synthesis to begin with. In 9 out of these 10 the hippuric acid output was raised by glycine feeding; in 5 cases by 0.3 gm. or more, *i. e.*, more than had been seen in the normal control subjects. In 6 out of these 9 the increase led to a normal level of hippuric acid excretion (0.85 gm. or higher).

In the second line of Figure 2 there are 4 control cases of individuals with faulty dietary habits. All suffer from anorexia of a psychologic origin, one was a textbook case of anorexia nervosa. One of these patients showed a finding unique among the entire investigated group, *i. e.*, a depression of the hippuric acid excretion during glycine administration.

Discussion. From these experiments it is obvious that in most senile cases the impairment of hippuric acid synthesis is only partly or not at all due to faulty conjugation. Otherwise we could not explain why exogenous glycine could improve the mechanism. In fact, 2 cases with conspicuously low values were raised to normal; this is particularly interesting because in animal experiments with N isotopes exogenous glycine, even when fed

in excess, supplied only one-third of the hippuric acid glycine (Rittenberg and Schoenheimer⁴). However, it was not possible to explain what factor determines the various patients' individual patterns. An attempt was made to correlate the difference between the glycine-free days and the 2nd day with various data such as the patient's weight-height index, dynamometer readings, and diet history; but no such correlation could be found.

However, although no correlation could be found with the patients' diet history, the phenomenon seems nevertheless to reflect on the nutritional state; for we saw a similar pattern in those otherwise healthy young individuals with anorexia (Fig. 2, Case SC and McC). In fact, the increase produced by glycine feeding in those cases is remarkable. Two values were raised by 0.67 and by 0.71 gm. respectively. Among these cases, however, there was one with a peculiar inverted reaction; in this case (CAN) the hippuric acid excretion was depressed with the administration of glycine. Errors in the collection or determination were excluded. It is impossible to explain this phenomenon but it is noteworthy that the same patient,

TABLE 1.—HIPPURIC ACID VALUES

No.	Name	Sex	Age	Wt. (lb.)	Ht.	Diagnosis	Average 1st + 3rd test of hippuric acid (gm.)	Deviation between 1st + 3rd test (gm.)	Hippuric acid (gm.) excretion following glycine adminis- tration	Increase (gm)
1	HI	M	28	186	6'3"	Normal	1.39	±0.01	1.30	-0.09
2	ME	F	30	120	5'4"	Normal	1.04	±0.03	1.32	+0.28
3	BO	M	29	200	6'1"	Normal	1.40	±0.05	1.62	+0.22
4	RO	F	26	130	5'4"	Normal	1.10	±0.10	1.36	+0.26
5	NO	M	27	168	6'	Normal	1.10	±0.02	1.36	+0.26
6	CR	M	24	150	6'	Normal	1.25	±0.01	1.39	+0.14
7	SC	F	29	85	5'1"	Anorexia nervosa	0.81	±0.09	1.52	+0.71
8	McC	F	56	86	5'1"	Anxiety state with anorexia	0.83	±0.01	1.50	+0.67
9	CAN	F	33	92	5'2"	Agitated depression	1.40	±0.01	1.10	-0.30
10	MU	F	38	93½	5'1"	Obsessive neurosis with anorexia	0.93	±0.03	1.16	+0.23
11	HOF	F	84	142	5'2"	Paranoid psychosis	0.80	±0.03	1.04	+0.24
12	CA	F	74	99	5'3"	Senile dementia paranoid	0.37	±0.07	0.84	+0.47
13	GRA	F	76	121	4'7"	Manic-depressive	1.01	±0.02	0.98	-0.03
14	GR	F	81	94	4'10"	Senile dementia	0.70	±0.02	1.06	+0.36
15	HO	F	58	101	5'1"	Pre-senile dementia	0.83	±0.08	1.20	+0.37
16	JOH	F	92	110	5'2"	Old schizophrenia with superimposed senile process	0.51	±0.11	0.85	+0.34
17	JO	F	64	107	5'	Senile dementia	0.43	±0.02	0.59	+0.16
18	LE	F	59	115	5'3"	Pre-senile dementia	0.91	±0.09	1.02	+0.11
19	MA	F	58	98	5'4"	Senile dementia	0.25	±0.05	0.49	+0.24
20	SH	F	80	109	4'11"	Senile dementia	0.57	±0.10	0.68	+0.11
21	SM	F	75	105	5'6"	Schizophrenia with superimposed senile process	0.40	±0.05	0.60	+0.20
22	CO	F	68	98	5'5"	Senile dementia	0.43	±0.03	1.03	+0.60

a lean asthenic woman who had been a very poor eater for some time, was gaining 6 pounds during the week in which the test was carried out. Even if we assume hypothetically that her N metabolism was in an anabolic phase during this

period it is difficult to explain why glycine added to the diet should not only not be used for conjugation but even depress that function. This would be contrary to the results of experiments with sodium benzoate feeding of growing animals

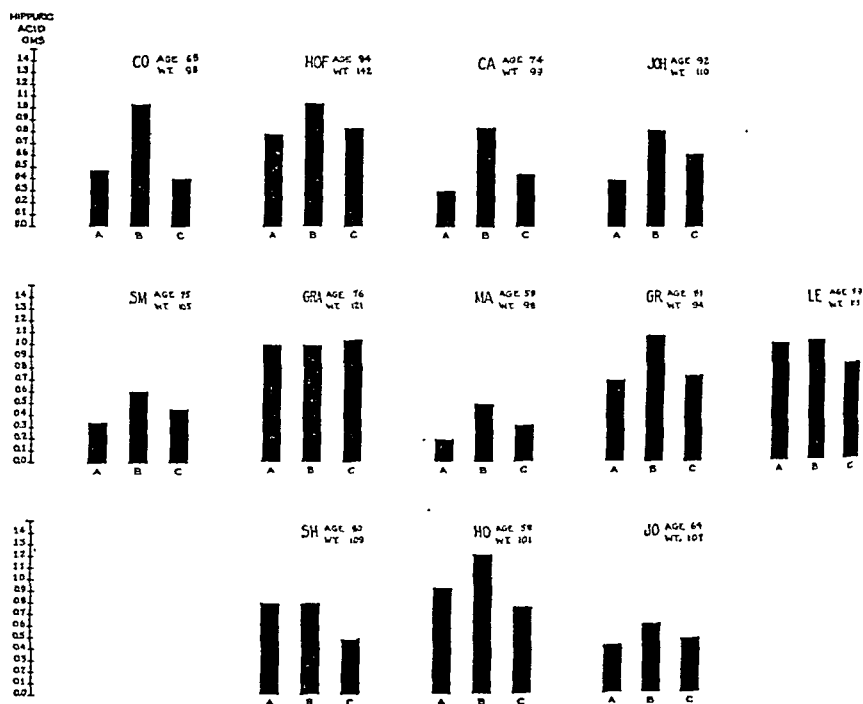


FIG. 1.—Senile and presenile cases. The hippuric acid excretion (in gm.) on 3 consecutive days is represented by 3 columns. A = 1st day, B = 2nd day, C = 3rd day. On the 2nd day (B) glycine was administered orally before and during the test (see test).

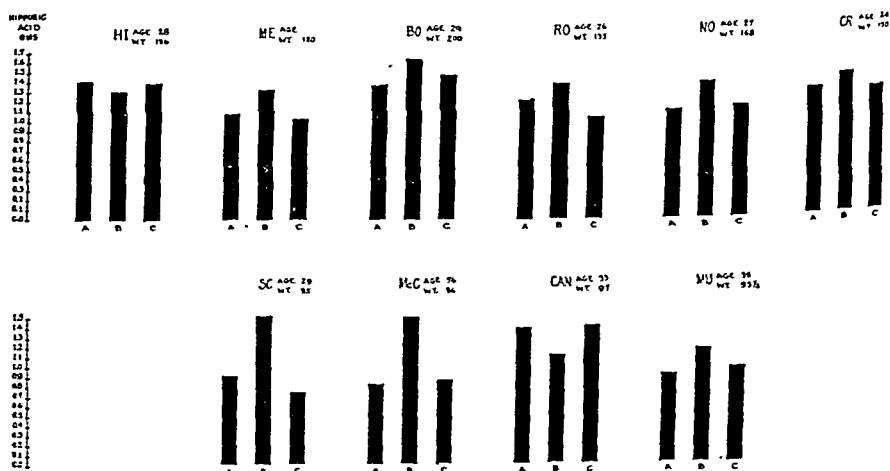


FIG. 2.—Control cases. The graphic presentation is the same as in Figure 1. The cases on the upper line are healthy young people. The cases on the lower line are patients with anorexia of psychologic origin, and debility, but otherwise healthy.

(White⁶). Probststein and Londe¹ found similar cases among their patients with impaired liver function, and attempted to explain this seemingly paradoxical finding by various hypotheses on glycine metabolism.

Summary. Hippuric acid synthesis was studied in 12 senile patients on 3 consecutive days; on the 2nd day glycine was administered orally before and during Quick's intravenous test. Six healthy adults and 4 adults with anorexia of psychologic origin served as control cases.

Ten of 12 senile cases showed an abnormally low hippuric acid synthesis to begin with. In 9 out of these 10, hippuric acid synthesis was increased to a varying degree by glycine administration. From this finding it can be concluded that faulty hippuric acid synthesis in old age is mainly due to an impairment of the organism to furnish glycine rather than to an impairment of the mechanism of conjugation. The difference in the patterns of these cases could not be correlated to any known clinical feature.

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THE ACTIVITY OF PENICILLIN IN THE INTRANASAL PNEUMOCOCCUS INFECTION OF MICE*

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THE method of infection of mice by inhalation had been thoroughly studied by Stillman and his co-workers¹² in the years 1924-1931, and excellent investigations as to the pathology and pathogenesis of the experimental pneumonia were contributed by Webster and Clow,¹⁴ and Rake.⁷ From these studies might be seen that the natural resistance of mice towards the intranasal infection is considerable and that neither all strains of pneumococci nor all breeds of mice were suitable for infections of this type. It was probably the finding of Stillman and Branch¹³ that treatment with high doses of alcohol was apt to break the natural and acquired resistance which induced Neufeld and Etinger-Tulczynska⁶ to adopt the method of short ether anesthesia preceding the nasal infection. By this modification, very consistent and reliable infections could be obtained.

The reason why the intranasal infection with pneumococci and the subsequent pneumonia of mice was not more widely used in the determination of antipneumococcal activity might probably be found in the fact that satisfactory infections require suitable strains of pneumococci and a susceptible breed of mice; another reason might be that most of the known chemotherapeutic agents exert only a very poor activity in this infection. We were unable to find any reference as to the

activity of sulfonamides in the pneumonia of mice; our own experience, covering roughly 10 years during which the intranasal infection was routinely studied with all agents which exerted antipneumococcal activity towards intra-abdominal infection of mice, showed clearly that in the group of sulfonamides, only exceptionally was a satisfactory activity found. Even in these cases, the therapeutic effect was generally not more than prolongation of life dependent on the duration of the treatment. Final survivors were observed merely occasionally.

From the observations of Gross and Cooper,² Kepl and Gunn,^{3,4} Raiziss, Kolmer and Rule,⁸ and Loughlin, Bennett, Flanagan and Spitz,⁵ who used the intratracheal and intrabronchial pneumococcus infection of rats, may be seen that the pneumonia of these animals responded to a certain degree to treatment with different sulfonamides and sulfones. Wood¹⁵ described particularly good results with sulfapyridine alone, but most of the other investigators^{3,4,5} agree that the combination with antipneumococcus serum enhances considerably the activity of the chemotherapeutic agents.

It seemed from these observations that the slow and predominantly bacteriostatic activity of the sulfonamides was not always sufficient to control the pulmonary infections of mice and rats. The different

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mechanism of activity and the more powerful antibacterial action of penicillin gave reason to expect a more definite effect in pulmonary infections.

Methods. Two strains of pneumococci were used in our experiments. One was a pneumococcus Type 1, obtained from the School of Hygiene of the University of Toronto. The strain came originally from the laboratories of the Rockefeller Institute. The second strain (Pn. 6302) was a Type 2 strain, obtained from the American Type Culture Collection. Both strains were kept on blood agar and in serum broth and were passed through mice regularly.

The mode of infection was simple: white mice* of 18 to 22 gm. were exposed to ether for 2 minutes, and then their noses were dipped into undiluted overnight serum broth of the pneumococci.

Untreated mice died within 30 to 48 hours after the infection. Pneumococci could be detected in the lungs by culturing on blood agar immediately after the infection. After 1 to 2 hours, a heavy infection of the blood was present. Pathologic changes, mostly dark red patches of bronchopneumonic infiltration, were generally not present before 24 hours; but at the time of death, both lungs were hepatized, of dark red color, and filled with exudate.

Treatment with penicillin was administered 1 hour after the infection. In some instances, a single treatment was given. No more than 4 treatments were found necessary, at least not with the doses selected for these experiments. The penicillin used in these studies was sodium salt of penicillin

prepared in the Roche research laboratories, its potency as determined by the cup test method was 500 to 800 u./mg.

The succumbed mice, treated as well as untreated ones, were autopsied, and cultures of the lungs and heart blood were taken. Surviving animals were under observation for a period of 3 weeks. From time to time, surviving mice were sacrificed and their lungs examined histologically.

Experimental. It is known by the work of Rake, McKee and their co-workers⁹ that very small doses of intra-abdominally injected penicillin were sufficient to control an intra-abdominal infection with pneumococci. Welch, Chandler, Davis and Price,¹⁵ on the other hand, found that an average of 478 u. injected intra-abdominally were required in order to protect 50% of mice infected with 10,000 m.l.d. of pneumococci. We consider 50 to 60 u./20 gm. as the minimal active subcutaneous dose for the control of an intra-abdominal infection with 1000 m.l.d. of pneumococci Type 1, Type 2 and Type 3.¹ Infections with Type 1 pneumococci sometimes responded to a total dose of 40 u./20 gm.

For the treatment of intranasal infections, higher doses were required.

In Table 1, the results of the subcutaneous treatment of intranasal infection with Type 1 and Type 2 pneumococci are given. A total dose of 800 to 1000 u./20 gm. can be considered the minimal active dose giving 50% survivors in the Type 1 infection, while about 1800 to 2000 u./20 gm. were required in infections with the Type 2 pneumococcus. Smaller doses, *c. g.*, 200 to 500 u./20 gm., showed still

TABLE 1.—ACTIVITY OF SUBCUTANEOUS TREATMENT WITH PENICILLIN IN PNEUMOCOCCUS PNEUMONIA CAUSED BY TYPE 1 AND TYPE 2 PNEUMOCOCCI

Total dose (u./20 gm.)	Type 1			Type 2		
	No. mice	No. survivors	% survivors	No. mice	No. survivors	% survivors
25-50	12	1	8 3			
100-150	12	3	25 0			
200	20	8	40 0			
400-500	24	10	41 0	15	0	0
800-1000	24	12	50 0	30	2	6 6
1600	13	9	69 0	5	0	0
1800-2000	60	27	45 0
3000-4000	5	5	100 0	19	15	79 0
Controls	40	1	2 5	75	2	2 6

* Strain of Tumblebrook Farms, Brant Lake, New York.

an appreciable activity in Type 1 infections, but the results with these doses were not always consistent.

The data for Type 1 infections represent the results of single treatment with high doses as well as those obtained by 2 to 4 times repeated treatment with smaller doses. As a rule, higher activity was observed with the latter method. In Type 2 infections, only repeated treatment was given.

In an attempt to analyze these observations, mice were killed 1, 3, 5, 9 and 25 hours following intranasal infection and a single treatment with 1000 u./20 gm. Cultures taken from the lungs showed that the number of pneumococci diminished gradually; after 9 hours, only a few scattered colonies could be recovered. After 25 hours, however, heavy growth from the lungs was again obtained in the cultures. If, however, 2 treatments of 1000 u./20 gm. were given with an interval of 4 hours, the lungs were sterile after 9 hours and stayed sterile if examined 24 hours after the treatment. This finding might explain why a single treatment, though it might be successful in some instances, was generally not sufficient; for the sterilization of the lungs, at least 2 treatments were necessary.

Pathologic findings in surviving treated mice were generally absent. During the first 3 or 4 days after the infection, very small marginal patches of pneumonic infiltration were occasionally found; but later, the lungs appeared normal by gross and microscopic examination.

Similar results were obtained when penicillin was given orally. Table 2 is meant to demonstrate the comparison of

subcutaneous and oral treatment in intranasal infections with pneumococcus Type 2.

In both groups of experiments, the same doses of penicillin were found to be active.

The observation that high total doses of penicillin (about 2000 u./20 gm.) had approximately the same effect whether the drug was given parenterally or orally might appear surprising. Experimental and clinical experience suggested that—as a rule—oral treatment required higher doses than the parenteral route. The observations in the intranasal pneumococcus infection were not the only and not the first exception to this rule.¹¹

The determination of the penicillin concentration in the blood seemed to offer some explanation for these findings.

The technique used for the determination of the serum level of penicillin was the Rammelkamp method.¹⁰

Groups of 5 mice were used for every dose and for every interval. The animals were sacrificed after the subcutaneous or oral administration by bleeding them from the axillary artery in ether anesthesia. The blood was pooled; and after clotting, the serum was used for the determination of potency.

The figures in Table 3 represent averages of 2 to 4 determinations with different groups of mice.

From the data on Table 3, it might be seen that in all dosage ranges, the peak of the concentration of penicillin in the serum was at least 10 to 20 times higher after subcutaneous than after oral administration. By increasing the subcutaneous dose, a longer duration of comparatively low concentrations was observed; however, the blood level dropped considerably

TABLE 2.—COMPARISON OF SUBCUTANEOUS AND ORAL ADMINISTRATION OF SODIUM PENICILLIN IN INTRANASAL INFECTIONS OF MICE WITH TYPE 2 PNEUMOCOCCI
(2 to 4 Treatments)

Total dose (u./20 gm.)	Subcutaneous			Oral		
	No. mice	No. survivors	% survivors	No. mice	No. survivors	% survivors
500-1000	20	2	10	40	2	5
1500-2000	60	27	45	84	38	45
3200-4000	5	3	60	50	29	58
7200	5	4	80
Controls	125	4	3			

within 2 hours in all dose ranges. The oral administration caused much less spectacular peaks and, accordingly, a more consistent serum level was found for a certain period of time, depending on the dose. Particularly after high doses (1000 u. and more), the serum level after oral treatment was more or less comparable to that of the subcutaneous dose in the later phases (after 2 to 3 hours), and was even occasionally found to be higher, *e. g.*, at the 3 to 5 hour intervals.

given to mice subcutaneously or orally, and the animals were infected intranasally with Type 2 pneumococci shortly afterwards and after intervals of 1, 3 and 5 hours. The results are given in Table 4.

This table shows that the effect of high doses of penicillin was the same whether the drug was administered subcutaneously or orally. If one considers not only the percentage of final survivors after 3 weeks of observation but also the survival time of the animals which died of pneumococcal

TABLE 3.—CONCENTRATION OF PENICILLIN IN THE SERUM OF MICE AFTER A SINGLE SUBCUTANEOUS AND ORAL TREATMENT WITH SODIUM PENICILLIN

Dose (u./20 gm.)	Route	Interval (minutes) after administration								
		15	20-30	60	90	120	150	180	210	300
100	Subcut.	2.0	1.15	0.25	0.06					
	Per os	..	0.075							
500	Subcut.	25.0	6.60	1.00	0.15	0.015	0.06	0		
	Per os	.	0.38	0.10	0.03	0.02	0.03	0		
1000	Subcut.	22.5	22.50	1.00		0.50	0.03	0.03		
	Per os	2.0	0.50	0.08	0.10	0.03	..	0.06	0.015	
2000	Subcut.	22.5	22.50	4.00		0.50	0.03	0.03	0	
	Per os	1.0	2.50	0.58	0.13	0.13	..	0.13	0 06	
5000	Subcut.	128.00	1.00	..	0.06
	Per os	2.00	1.00	..	1.00

TABLE 4.—PROPHYLACTIC ACTIVITY OF A SINGLE DOSE OF SODIUM PENICILLIN AFTER SUBCUTANEOUS AND AFTER ORAL ADMINISTRATION AGAINST INTRANASAL INFECTION OF MICE WITH TYPE 2 PNEUMOCOCCI (EXPERIMENTAL PNEUMONIA)

Penicillin (u./20 gm.)	Route of administration	Interval* (hours)	No. mice	Average survival time (days)	% survivors
2500	Subcut.	0	9	4.0	45 0
		1	29	4 3	41 5
		3	27	2 3	22 0
	Per os	0	10	4.0	80.0
		1	30	7 0	40 0
		3	35	5 6	31 0
	Subcut.	1	15	3 0	56 0
		3	20	2 3	20 0
		5	15	2 4	14 0
5000	Per os	1	10	9.0	60 0
		3	20	6 6	25 0
		5	15	7 0	20 0
	Controls	..	48	2 0	2 5

* Interval between treatment and infection.

The question arose whether these differences in the blood level were of therapeutic significance. A suitable way of obtaining information to this point seemed the investigation of the prophylactic activity of large single doses of penicillin.

Doses of 2500 and 5000 u./20 gm. were

pneumonia during this period, it might appear that the oral prophylactic dose was even somewhat more effective.

Similar results were obtained in intra-abdominal streptococcal infections; these experiments will be published in the near future.

Discussion. The intranasal infection of mice with Types 1 and 2 pneumococcus which produced fatal pneumonia and septicemia responded readily to subcutaneous treatment with penicillin. Comparatively high doses, 1000 u. (Type 1) to 2000 u. (Type 2), were required to control the infection in approximately half of the treated animals. Higher doses gave a correspondingly higher percentage of survivors.

The observation that the oral administration of penicillin was as effective as the parenteral one was in agreement with our earlier findings¹¹ in infections with meningococci, *Salmonella* and *Borrelia noryi*. In these infections which required high doses of penicillin (200 u. and more), the ratio

$$\frac{\text{Minimal oral dose}}{\text{Minimal subcutaneous dose}}$$

was small and did not exceed values of 1 to 2. On the other hand, in infections which responded to comparatively small doses of penicillin, *e. g.*, the intra-abdominal streptococcal and pneumococcal infections, the ratio was about 10. In more severe infections or in infections with more resistant organisms which required higher doses of penicillin by the parenteral route the ratio oral/subcutaneous dose showed a tendency to drop. The comparison of intra-abdominal pneumococcus infection (ratio 8 to 10¹¹) and intranasal infection (ratio 1) is only one example; the same relation was found in streptococcal infections. While, for instance, the ratio oral/subcutaneous dose was 10 in infections with 1000 m.l.d., it dropped to 1.5 if 1,000,000 m.l.d. of the same streptococcus strain were used for the infection.

The determination of the blood level seemed to offer an explanation for these findings, because in the later intervals after high doses of penicillin, the concentrations of penicillin in the serum were almost identical whether the drug was given per os or subcutaneously. That would mean that the very high initial peak which was only observed after parenteral administration was of lesser significance than the consistent level of comparatively low con-

centrations to be found in the serum during the hours following the subcutaneous as well as the oral administration.

From the prophylactic and therapeutic experiments in mice, we might draw the conclusion that there was no decisive influence of the peak concentration after high doses of penicillin. The blood level reached after the oral doses was obviously sufficient not only to protect mice against the intranasal infection but also to prevent septicemia and death if the treatment was started 1 hour after the infection.

Whether these findings have some bearing on the penicillin therapy of infections in humans cannot be decided at the present moment because only scanty information as to the dosage and effectiveness of oral penicillin treatment in different types of infections is available. We have, however, the impression that the oral route of penicillin administration based on an adequate dosage schedule might be useful in all conditions where a consistent blood level has to be maintained over a longer period of time.

Summary. 1. Penicillin exerted a very definite effect in the prevention of pneumonia of mice caused by intranasal infection with the pneumococcus Type 1 and Type 2.

2. The minimal active total dose in Type 1 infections was 800 to 1000 units per 20 gm. given subcutaneously in 2 to 4 treatments, while Type 2 required a total dose of 1800 to 2000 u.

3. In intranasal pneumococcus Type 2 infection of mice, oral administration of penicillin was as effective as the subcutaneous route. This was found not only if penicillin was given 1 hour after the infection but also in prophylactic experiments when the mice were infected 1 to 5 hours after the administration of penicillin.

4. The determination of the penicillin level in the serum of mice seemed to suggest that the concentrations obtained after high oral doses were sufficient to control the intranasal pneumococcus infection, although they did not produce as high an initial peak of the serum level as observed after parenteral injection.

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SUCCINYLSULFATHIAZOLE IN THE NUTRITION OF THE RAT

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It has been reported that rats receiving succinylsulfathiazole in *stock* or *natural* diets grow normally and show no evidence of nutritional deficiencies.^{8,9} In contrast, the inclusion of succinylsulfathiazole in the usual adequate *highly purified* diets renders such diets incapable of supporting normal growth. The feeding of these diets leads to the development of a variety of deficiency signs including alopecia, achromotrichia, porphyrin-stained whiskers, leukopenia and agranulocytosis.^{7,9,12} These outward manifestations of nutritional deficiencies are accompanied by decreased hepatic storage and fecal elimination of folic acid, biotin and even pantothenic acid. The administration of folic acid and biotin will restore such animals to normalcy with respect to both appearance and hepatic vitamin storage.¹²

Although no apparent detrimental effects result from the feeding of succinylsulfathiazole to rats on stock diets, it should be recognized that it is quite possible for an animal to appear normal, but to possess a tissue vitamin storage indicative of a latent or borderline deficiency. Consequently these additional growth studies have included determinations of the hepatic storage and fecal elimination of folic acid, biotin and pantothenic acid when rats were fed succinylsulfathiazole in a complete diet. Fecal *Escherichia coli* counts also have been made as a measure of the bacteriostatic effectiveness of the drug.

Procedure. Thirty-two weanling male albino rats were separated into 2 equivalent groups and were caged individually over wide-mesh screening. They were given food and water *ad libitum*. Group 1 received a diet of 95% powdered "Friskies" and 5%

Wilson's 1 to 20 liver powder. Group 2 received a diet composed of 90 parts of the above ration and 10 parts of succinylsulfathiazole. The ratio of succinylsulfathiazole to calories consumed in this diet is approximately 3 times that existing when succinylsulfathiazole is administered in its usual dosage to human patients. On a gm./kilo/day basis the rats ingested 20 to 40 times the recommended human dose. The experiment was of 8 weeks duration and the rats were weighed at weekly intervals. Occasional food intake determinations were carried out on representative rats throughout the experiment. At intervals vitamin determinations also were carried out on complete 24 hour fecal collections. The fecal *E. coli* counts were made on representative rats after they had consumed the diet for 8 weeks. At the conclusion of the experiment all of the animals were killed by decapitation and representative livers were assayed microbiologically for the various B vitamins studied.

The preparation of the fecal and liver samples for microbiologic assay has been described previously.¹¹ Folic acid was determined microbiologically with *Lactobacillus casei* as the assay organism.² Biotin, pantothenic acid and nicotinic acid were determined with *Lactobacillus arabinosus*.^{5,6,10} Ten mg. of para-aminobenzoic acid per 100 ml. were included in the assay medium as a sulfonamide inhibitor in the microbiologic assay of feces. A folic acid concentrate was used as a standard in the microbiologic assay of folic acid. The standard has been assayed against a sample of synthetic *Lactobacillus casei* factor, supplied through the courtesy of the Lederle Laboratories, and the results have been recalculated in terms of the presumably pure factor. The *E. coli* counts were obtained by a dilution plate count method using desoxycholate agar as a differential medium.

Results and Discussion. The data obtained have been summarized in Table 1.

The inclusion of 10% succinylsulfathiazole in the adequate non-synthetic diet employed did not alter significantly the growth or normal appearance of the rats. The rats consuming the succinylsulfathiazole-containing diet ingested significantly more food even when the food intakes were corrected for the fact that only 90% of the diet was nutritive material. The average amount of feces produced per day by the rats consuming the sulfonamide diet was a little higher than that produced by the rats on the control diet. The difference was not statistically significant, however, probably because of the great variability in daily feces production that exists in rats.

The fecal elimination of folic acid was significantly depressed in the rats consuming the succinylsulfathiazole diet. Miller has shown, *in vitro*, that sub-bacteriostatic amounts of sulfanilamide markedly decrease the amount of folic acid synthesized by *E. coli*.³ Probably this phenom-

non occurs also with other intestinal organisms and other sulfonamides. In the present experiment the fecal elimination of biotin, pantothenic acid and nicotinic acid was not significantly altered by the administration of succinylsulfathiazole.

The hepatic storage of folic acid was found to be 5 (4.1-6.2) γ /gm. in the control animals and 2.5 (2-3.5) γ /gm. in the sulfonamide-fed group. This difference is statistically significant. Previous studies have shown that the folic acid content of the liver of rats fed *natural* diets may range from about 4-12 γ /gm.^{4,12} When rats are reared on *highly purified* diets which furnish no external source of folic acid to the rat, but which are entirely adequate for normal growth, the animals are able, probably by bacterial synthesis in the intestine, to build up an hepatic storage of folic acid in the order of 0.6-1.5 γ /gm. The inclusion of succinylsulfathiazole in the diet reduces this value to 0.2-0.6 γ /gm. and deficiency symptoms

TABLE 1.—SUMMARY OF EXPERIMENTAL DATA

	Control group*	Succinylsulfathiazole-fed group*	P†
Initial weight, gm.	59 (54-66) (16)	60 (56-68) (16)	0 1-0 2
Final weight, gm.	236 (214-276) (16)	231 (188-271) (16)	0 5-0 6
Food intake, gm./day . . .	16 8 (14-20) (36)	20 5 (15-24) (36)	<0 01
Corrected food intake, gm./day	18 5 (13 5-21 6) (36)	<0 01
<i>E. coli</i> organisms in feces, No./gm. .	400,000 (128,000-900,000) (3)	270 (20-500) (3)	
Feces produced, gm./day . . .	9 7 (7 6-12 5) (5)	12 7 (8 6-19 7) (6)	0 1-0 2
Fecal elimination of:			
Folic acid, γ /day . . .	31 (26-41) (5)	17 (9-26) (6)	<0 01
Biotin, γ /day	5 3 (3 3-6 7) (5)	4 3 (3 8-4 9) (5)	0 2
Pantothenic acid, γ /day . .	416 (311-484) (5)	339 (232-430) (6)	0 05-0 1
Nicotinic acid, γ /day . . .	525 (463-576) (3)	507 (474-552) (3)	0 6-0 7
Hepatic storage of:			
Folic acid, γ /gm.	5 0 (4 1-6 2) (8)	2 5 (2 0-3 5) (8)	<0 01
Biotin, γ /gm.	0 93 (0 82-1 0) (8)	0 83 (0 77-0 91) (8)	0 02-0 05
Pantothenic acid, γ /gm. . .	60 (52-66) (8)	65 (52-75) (8)	0 2

* Accompanying the average values presented are (1) the range in values obtained, and (2) the number of animals involved or determinations performed.

† The P values or significance were calculated by the *t* test where

$$t = \frac{x_1 - x_2}{\sqrt{e_1^2 + e_2^2}} \quad \epsilon \text{ for 30 or more observations} = \frac{\sum x_i^2}{n^2} - n\bar{x}^2$$

$$\epsilon \text{ for less than 30 observations} = \frac{\sum x_i^2}{n(n-1)} - n\bar{x}^2$$

$$\text{Degrees of freedom} = n_1 + n_2 - 2$$

We are indebted to Mr. Joseph L. Ciminera for the statistical calculations.

are encountered. The hepatic storage of folic acid found in the present sulfonamide-fed group was 2 to 4 times that found in rats reared on purified diets and 5 to 10 times that found in rats actually showing evidence of a folic acid deficiency. It should be noted that folic acid is unique among the B vitamins in that the liver storage ordinarily found in stock rats is approximately 20 times the level found in deficient rats. In contrast, pantothenic acid, riboflavin, or biotin-deficient rats have an hepatic storage of the limiting factor that is one-third to one-half that found in stock rats.

Despite the long-continued feeding of succinylsulfathiazole the coliform organisms were essentially absent from the feces at the conclusion of the experiment. Results have been reported in which *E. coli* organisms reestablished themselves following administration of succinylsulfathiazole for a period of several weeks.¹ The sulfonamide was fed at a level of 0.5% and it is possible that this concentration permitted sulfonamide resistant strains to develop and become reestablished in the intestine.

In the present experiment leukocyte counts were not made. It has been our experience that low leukocyte counts and agranulocytosis do not occur independent

of a depression in growth or a decrease in the hepatic storage of folic acid below a level of about 0.8 γ /gm.

Summary. 1. Succinylsulfathiazole was administered to growing rats over an 8 week period in a dose which, in comparison with the prescribed human dose, was approximately 3 times greater on the basis of % of food intake and 20 to 40 times greater on the basis of gm. per kg. of body weight.

2. The growth, general appearance, hepatic storage of biotin and pantothenic acid and fecal elimination of biotin, pantothenic acid and nicotinic acid were not significantly influenced by the ingestion of the sulfonamide.

3. The animals consuming the sulfonamide-containing diet eliminated significantly less folic acid in the feces and at the conclusion of the experiment were found to have significantly less folic acid stored in the liver. The hepatic storage of folic acid was, however, approximately 3 times that found in rats receiving adequate highly purified diets and 8 to 10 times that found in folic acid deficient animals.

4. The *E. coli* organisms were practically eliminated from the feces of the succinylsulfathiazole-fed rats.

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EFFECT OF CITRATE ON THE REACTIONS OF LEAD WITH BLOOD CELLS AND PLASMA*

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In papers dealing with the use of sodium citrate as an agent in the treatment of plumbism, Kety and Letonoff^{8,9} presented evidence that the administration of the citrate results in the prompt reduction of the concentration of lead in the blood and, in most cases, an increase in its excretion. These observations were subsequently confirmed by Cantarow and Trumper.⁶ Previously, Aub and co-workers¹ noted a slight increase in the rate of lead excretion following sodium citrate administration.

It has long been known that citrate solutions dissolve certain relatively insoluble lead salts, an action which has been attributed to the formation of a soluble lead citrate complex. Proof of the existence of such a complex in dilute solutions was obtained by Kety,⁷ who concluded that even the normal plasma citrate may be responsible for keeping otherwise insoluble lead compounds in solutions and, hence, for their physiologic removal from the blood. This conclusion has been questioned, however, by Bambach, Kehoe and Logan³ on the basis that lead is carried mainly in the cells.

In view of these observations, it seemed of interest to investigate further the mechanism by which citrate influences lead metabolism. The present paper deals with the effect of citrate on the combination of lead with cells and plasma, as indicated by changes produced in: (a) the plasma cell partition; (b) the ability of the cells to remove lead from the plasma; and (c) the proportion of diffusible lead in the plasma as determined by ultrafiltration. Any process that alters these factors might reasonably be expected to affect the mobilization and excretion of lead.

Methods. All experiments were performed with freshly drawn human blood *in vitro* at a temperature of 37.5° C. (*In vivo* studies will be the subject of a subsequent paper.) Heparin was used as the anticoagulant. Solutions of lead chloride and sodium citrate were added in small amounts to the blood or other fluid under investigation by means of micropipettes, so that the total increase in volume did not exceed 2% of the volume of the specimen.

Lead analyses were made by using radium D, an isotope of lead, as a radio-active indicator and measuring the activity with a Geiger-Müller counter, as previously described.¹¹ While the values obtained by this method represent added lead only, they roughly approximate the total amounts under the conditions employed in this work, where the quantity of lead initially present was always small in comparison with that added. For the experiments involving the addition of relatively small amounts of lead, blood specimens having an initial concentration of less than 0.01 mg. per 100 cc., as found by the dithizone method, were selected. The concentration of lead in the cells was calculated from the hematocrit value and from the concentrations in whole blood and in plasma, except in the few cases in which the cells, after separation from the plasma, were suspended in saline and analyzed directly.

No attempt was made to determine the initial citrate content of the blood specimens. Normal plasma contains from 1.5 to 4 mg. per 100 cc., according to Schersten,¹³ but as much as one-fifth of the amount contained in a sample may disappear within 1 hour.¹² All concentrations given in the present paper refer to added citrate only.

Ultrafiltrates were obtained by means of a filtering system constructed of glass vessels and a membrane of dialyzer cellophane which had been soaked in several changes of distilled water to remove the glycerol. The

* This investigation was aided by a grant from the Alumni Research Foundation of the College of Medical Evangelists.

solution and filtrate could come in contact with nothing but glass and the membrane at any point. A positive pressure of about 450 mm. of mercury was employed. With this apparatus, ultrafiltrates of plasma and serum gave negative coagulation tests with heat and acetic acid.

Results. Plasma Cell Partition. After preliminary tests, which indicated that in some instances sodium citrate alters the distribution of lead between the plasma and cells, the effect of various concentrations of citrate with different levels of lead was investigated. In the first experiment, graded amounts of the citrate were added to 2 cc. samples of blood. Active lead chloride was then introduced, so as to give a predetermined lead concentration. After standing for 20 minutes with occasional gentle inversion, the mixtures were centrifuged, and samples of plasma were analyzed for lead. Typical results are presented in Figure 1A. With a total lead concentration of 0.1 mg. per 100 cc., less than 4% of the lead remained in the plasma, and the addition of citrate had only a slight effect on the amount found in this fraction of the blood. At the level

of 0.2 mg. per 100 cc., the proportion of lead in the plasma of untreated blood was greater, but was considerably reduced by the presence of the citrate.

In this connection, it may be pointed out that in clinical and experimental lead poisoning, more than 90% of the element is held by the cells.^{3,11} A similar distribution is usually found when soluble lead salts are mixed with blood *in vitro*, provided the concentration is kept comparatively low, *e. g.*, 0.1 mg. per 100 cc. However, the present results show that at higher levels, a higher, but variable, percentage of lead may be found in the plasma, the actual percentage apparently depending upon the manner of mixing, as well as upon individual variations in blood specimens. It is at the higher lead levels that the effect of citrate in facilitating the uptake by the cells becomes most evident.

A blank experiment was performed by repeating the procedure with samples of plasma instead of whole blood. In every case the concentration of lead was the same in the upper and lower portions of the plasma after centrifuging, thus indicating that the disappearance of lead from

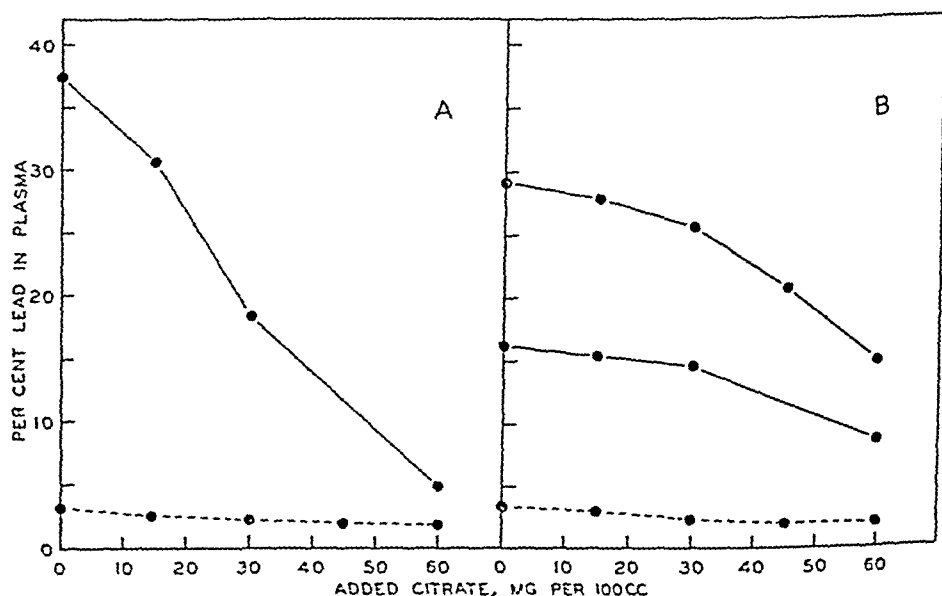


FIG. 1.—The effect of citrate on the distribution of lead between cells and plasma. Solid lines represent a lead concentration of approximately 0.2 mg. per 100 cc.; dotted lines indicate 0.1 mg. per 100 cc. A, Lead added to blood containing citrate. B, Blood treated with citrate after the addition of lead.

the plasma, with increasing amounts of citrate, was dependent upon reaction with the cells.

Figure 1*B* shows results obtained in the same way as those represented in Figure 1*A*, except that the lead was added to the blood first, and the mixture was allowed to stand for 20 minutes before the introduction of citrate. It will be noted that the citrate produces a smaller effect on the plasma cell partition when added to blood previously mixed with the lead.

The effect of the length of time elapsing between the addition of citrate and centrifugation was also studied. In one experiment in which sodium citrate was added to the blood with a lead concentration of 0.18 mg. per 100 cc., 14 and 5% of the lead was found in the plasma fractions at the end of 5 and 20 minutes, respectively, while in untreated controls the corresponding values were 34 and 32%. Very little change occurred after the first 20 minutes, irrespective of amount of citrate present.

Blood samples were also treated with solutions of certain other substances which have been advocated for use as "de-leading agents"—sodium bicarbonate, sodium thiosulfate, potassium iodide, disodium phosphate and ascorbic acid—but in no case was the distribution of lead measurably different from that in untreated controls.

remove considerably less of the element than could be expected from the results of introducing lead solutions directly into whole blood. According to the latter authors, these observations indicate the formation of some type of combination of lead and plasma. In the present work, a study was made of the influence of citrate on the ability of lead to thus combine with plasma.

Blood samples were treated with varying amounts of sodium citrate. The plasma was then separated, and mixed with active lead chloride. After standing for 10 minutes, it was returned to the cells. All samples had a final lead concentration of approximately 0.1 mg. per 100 cc. At the end of 20 more minutes, the mixtures were centrifuged and the two blood fractions analyzed for lead. The results (Table 1) show that under the conditions of these experiments, the presence of added citrate greatly increases the amount of lead transferred to the cells. While 33% remained in the plasma in the absence of citrate, all but 7%, for example, was removed by the cells in presence of 15 mg. of citrate per 100 cc. Thus the ability of the plasma to withhold from the cells, lead previously mixed with it, is greatly reduced by the addition of sufficient citrate.

TABLE 1.—EFFECT OF CITRATE ON THE ABILITY OF CELLS TO REMOVE LEAD FROM PLASMA

Sample	Citrate (mg./100 cc.)	Lead in cells (mg./100 cc.)	Lead in plasma (mg./100 cc.)	% lead in plasma
1	0	0 14	0 065	32
2	3	0 14	0 059	29
3	5	0 15	0 049	24
4	15	0 19	0 014	7
5	30	0 19	0 008	4

Combination of Cells With Lead in Plasma. Aub and co-workers² noted that lead mixed with serum could no longer produce its usual effects on the red cells. As stated by Minot,¹⁰ lead appears to be inactivated by admixture with serum. Bambach, Kehoe and Logan³ made the further observation that when cells are separated from plasma, and are returned after adding lead to the plasma, they

The influence of time on this action of citrate is shown in Table 2. Samples of the remixed blood, containing the same amount of lead as before and 15 mg. of citrate per 100 cc., were centrifuged at different periods of time after mixing. Other samples containing no citrate served as controls. As can be seen from the data, lead is taken up rapidly by the cells in the presence of citrate. It will also be

noted, incidentally, that the percentages of lead in the plasma are somewhat higher than the corresponding values obtained in the previous experiment (Table 1). Such variations were encountered when the specimens were collected at different times and from different individuals. Nevertheless, both sets of data clearly show the "activating" effect of citrate on lead mixed with plasma.

Combination of Cells With Lead in Ringer's Solution. In this experiment, washed cells were mixed with lead and citrate dissolved in a special Ringer's solution containing phosphate, as used by Aub and co-workers.² All samples had a final lead content of approximately 0.1 mg. per 100 cc. One series of mixtures was centrifuged after 5 minutes, and another at the end of 30 minutes. As shown in

TABLE 2.—EFFECT OF TIME ON THE ABILITY OF CELLS TO REMOVE LEAD FROM PLASMA

Time after mixing cells and plasma (min.)	Without citrate			With citrate		
	Lead in cells (mg./100 cc.)	Lead in plasma (mg./100 cc.)	% lead in plasma	Lead in cells (mg./100 cc.)	Lead in plasma (mg./100 cc.)	% lead in plasma
2	0.038	0.16	81	0.15	0.051	26
10	0.067	0.13	67	0.18	0.025	12
30	0.082	0.12	59	0.18	0.020	10
60	0.088	0.11	56	0.18	0.019	9

Transfer of Lead From Cells to Plasma.

The reversibility of this reaction was also investigated. Results reported previously¹¹ showed that lead passes readily from cells to plasma in experimental animals. On the other hand, Blumberg and Scott⁴ failed to remove appreciable quantities of lead by washing cells twice with isotonic saline. In the present work, 3 samples of blood containing 0, 5 and 15 mg. of citrate per 100 cc., respectively, were allowed to stand until the cells had reached equilibrium with lead in the plasma. The cells were then centrifuged down, separated as completely as possible from the plasma, and washed 3 times for 5 minutes with an equal volume of fresh plasma containing no added lead. Analyses made on the plasma washings and on the washed cells revealed that the second and third washings had each removed from 2 to 3% of the lead, which was almost as much as could be expected from the results of distribution studies¹¹ on blood having a similar lead content (0.1 mg. per 100 cc.). The percentages of lead removed from the 3 samples were the same, however, within the limits of experimental error. It may be concluded, therefore, that the transfer of lead from the cells to the plasma is not appreciably affected by the presence of extra citrate.

Table 3, even a comparatively low concentration of citrate produced a large increase in the amount of lead taken up by the cells and, unexpectedly, higher concentrations proved to be somewhat less effective. In blank tests performed with similar concentrations, no sedimentation of lead occurred in the absence of cells.

Ultrafiltration. Plasma and serum samples were treated, in turn, with sodium citrate and lead chloride, and allowed to stand a few minutes, after which, the mixtures (containing 0.1 mg. of lead per 100 cc.) were subjected to ultrafiltration. Table 4 gives the concentrations of lead in the ultrafiltrates, expressed in terms of % of the original plasma and serum concentrations. Each value represents the average of 2 determinations made on successive fractions of ultrafiltrate. The data indicate that citrate increases the percentage of diffusible lead, as thus determined, in plasma and in serum.

Discussion. These actions of citrate appear to be possible factors in the increased mobilization and excretion of lead observed in sodium citrate therapy. The fact that citrate promotes the removal of lead from plasma and from Ringer's solution, suggests that it may aid in the mobilization of the element by increasing the capacity of the cells to combine with lead

from the tissue fluid. This factor, coupled with the increase in the proportion of the diffusible form produced by citrate, should result in a freer exchange of lead between the tissues and the blood. The increased diffusibility should also make more of the element available for excretion.

Citrate ion is known to exhibit a moderate indifference to cells. It is possible that its properties are so modified by the lead as to permit the complex to combine readily with the cells. However, it seems more likely that citrate ion serves as a carrier which takes up and then

TABLE 3.—EFFECT OF CITRATE ON THE ABILITY OF CELLS TO REMOVE LEAD FROM RINGER'S SOLUTION CONTAINING PHOSPHATE

Sample	Time before centrifuging (min.)	Citrate (mg./100 cc.)	Lead in cells (mg./100 cc.)	Lead in "plasma" (mg./100 cc.)	% lead in "plasma"
1	5	0	0 09	0 110	55
2	5	3	0 18	0 020	10
3	5	5	0 18	0 023	12
4	30	0	0 11	0 090	45
5	30	3	0 18	0 021	11
6	30	5	0 17	0 031	16
7	30	15	0 16	0 041	21
8	30	60	0 10	0 090	47

TABLE 4.—EFFECT OF CITRATE ON THE % OF DIFFUSIBLE LEAD IN PLASMA AND SERUM AS DETERMINED BY ULTRAFILTRATION

Citrate (mg./100 cc.)	Lead in plasma ultrafiltrate (%)	Lead in serum ultrafiltrate (%)
0	6	9
8	18	15
20	30	38

In considering these results in relation to therapy, it should be noted that the range of citrate concentrations employed in most of the experiments included some values which undoubtedly are considerably above those attained in the body. The administration of sodium citrate is known, however, to result in an elevation of blood citrate, and could be expected to influence the behavior of lead in the blood to a corresponding extent.

The experimental results are probably best explained on the basis of an increased reaction of citrate ion with lead ion to form the soluble complex,⁷ in competition with other plasma constituents which also combine with lead. Additional evidence for the presence of this complex in solutions containing lead and citrate ions has recently been obtained by Bobtelsky and Jordan.⁸ Although this explanation appears adequate to account for the observed reduction in the non-diffusible form in the plasma, the increased ability of cells to combine with lead may also involve other

releases lead ion to the cells. The latter view appears to find support in the observation that in Ringer's solution a relatively low concentration of citrate greatly increases the amount of lead taken up by the cells, while higher concentrations produce a smaller effect.

Summary. 1. The effect of sodium citrate on the distribution of lead between the plasma and cells, and on the proportion of diffusible lead in plasma and serum, has been investigated, using the isotope, radium D, as a radioactive indicator.

2. When lead is added to blood *in vitro* at such concentrations that a large fraction ordinarily remains in the plasma, the presence of added citrate increases the percentage of lead taken up by the cells.

3. The addition of citrate greatly increases the ability of the cells to combine with lead previously mixed with plasma or with Ringer's solution containing phosphate.

4. Lead is readily transferred from cells to plasma, but citrate has little or no effect on the process. of non-diffusible lead in plasma and serum, as determined by ultrafiltration.
5. Added citrate reduces the proportion of non-diffusible lead in plasma and serum, as determined by ultrafiltration. 6. The possible bearing of these results on citrate therapy is briefly discussed.

I am indebted to Miss Margaret Anderson for technical assistance.

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ALTERATION OF THE ELECTROCARDIOGRAPHIC P WAVES IN ACUTE RHEUMATIC FEVER

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ALTHOUGH many reports have been made of electrocardiographic changes in patients with acute rheumatic fever,^{8,10,12,13} there have been few detailed analyses of the frequency of various abnormalities or their changes during various stages of clinical activity of the disease. Wendkos and Noll¹² present a recent summary of most of the changes that occur during active rheumatic fever, including delay or deformity of the QRS complex, modification of the S-T segment and the T waves, as well as auriculoventricular conduction delay. During the 1943 epidemic of acute rheumatic fever at the United States Army Air Forces Station Hospital at Lowry Field, Colorado, these and certain other changes considered significant were observed. Since it has not received special comment previously, the modification of P waves observed in rheumatic fever is considered worthy of report.

It would be expected from the frequent extensive involvement of the atrial muscle that the electrocardiographic P waves would be modified during the active phases of acute rheumatic fever. It is believed that such changes commonly occur but that there has been hesitancy in either recognizing or reporting them because of the normal variations in form and direction of P waves, especially in Lead III. The respiratory cycle variations of P₃ is notably great.

Alterations of form, amplitude, duration and direction have not only been described in arrhythmias involving aberrations of atrial pacemaking and conduction, but likewise in mitral stenosis,^{2,11} and pulmonary valve stenosis.¹ There have also been studies of the P waves in esophageal leads,^{2,9} myocardial infar-

tion,^{7,9} and myocardial failure.⁵ Recently there has been a detailed study of the electrical axis of P waves in normal subjects and in those with atrial arrhythmias.^{4a,b,c} The variations in electrical axis seem to have been influenced more by the size and position of the auricles than by intrinsic changes in the myocardium affecting conduction.

P wave changes as herein reported in acute rheumatic fever cases are thought to be significant, especially when successive records show changes in form that seem to coincide with increased activity of the disease. Flattened, diphasic or inverted P₃ waves were not considered significant unless the abnormality was great enough to modify P₂ as well as P₃ and exhibit changing form on successive records. However, changes of lesser degree may be considered significant in future studies. The respiratory variation of P₃ was adequately discounted in evaluating the changes interpreted as abnormal. P₁ variations generally were accompanied by similar modifications of P₄ and of P₂ to an appreciable degree less frequently.

In 60 successive active acute rheumatic fever cases studied, out of 551 observed during the period of January 1943 through September 1943, 8 (approximately 11.7%) showed significant flattening or inversion of P waves. Five of these cases showed variations in successive records taken at weekly intervals and the flattening or inversion of these waves generally seemed to coincide with a period of activity, corroborated by clinical and laboratory evidences. Of the 8 cases, 5 presented flattening or inversion of P₂ and P₃; 2 demonstrated alterations in P waves in all leads including Lead IV F, and 1 showed only

P₁ and P₄ inversion. It is apparent from these cases that changes are more common in Lead III than in Leads I or IV F. In view of the greater prominence of auricular flutter waves in Lead III than in Lead I, probably due to the more perpendicular position of this lead to the average axis of the auricular vectors, it might be expected to reflect more sensitively the changes in auricular conduction. Auricular changes would likewise be expected in esophageal leads and such was demonstrated in 2 of the cases with abnormal P waves at 35 cm. and 40 cm. levels with loss of the initial upright phase of the sharp diphasic complex.

These alterations in P wave directions are apparently a sensitive index of cardiac involvement in that in only 2 out of the 8 cases were other unequivocal electrocardiographic abnormalities noted, namely a flattened T₁ in 1 case and prolongation of the auriculoventricular conduction period in another. Diphasic T₃, flattening of T IV F, low voltage of the QRS and right axis deviation occurred in 4 other cases but these changes could not be interpreted as definitely abnormal. Comment may be made that tendency toward right axis deviation was observed with unusual frequency in acute rheumatic fever patients, without evident valvulitis. No explanation is offered for this finding.

In addition to these 8 cases there were 7 others in the 60 studies in which minor P wave changes were observed but were not considered significant. Greater amplification of the waves may permit the development of more exact diagnostic criteria for such records, which now are not considered diagnostic.

Certain case histories serve to illustrate the relation of atrial changes in the electrocardiogram to the clinical activity of the acute rheumatic fever.

Case Histories. CASE 1. Patient X, age 32, was admitted to the Station Hospital, Lowry Field on Aug. 4, 1943, with pain, swelling, redness and tenderness of both ankles and the right knee of 3 days duration. He had noted fatigue, dyspnea on exertion, and anorexia for the past 2 months,

succeeding an upper respiratory tract infection. There was a past history of recurrent pains in both ankles and lower legs, between the ages of 6 and 9 years. On admission the oral temperature was 99.4° F., the pulse rate 100 per minute, the white blood count 8300 and the erythrocyte sedimentation rate 5 mm. per hour (Cutler technique). Roentgen films of the heart and lungs were normal. The electrocardiogram on Aug. 6, 1943, showed a low voltage in all leads, and a sharply inverted P₃ and slightly inverted P₄.

On August 19, at which time all joint signs had subsided and the temperature had returned to normal, the P₃ deflection had reverted to upright. The electrocardiogram on August 28 was unchanged except that voltage in all leads had increased slightly. The sedimentation rates repeatedly remained normal as did the leukocyte count. This case illustrates definite alteration of the direction of P₃ during clinical activity of acute rheumatic fever accompanied by low grade fever but no other laboratory evidence of activity of the disease.

CASE 2. (Figs. 1 and 2.) Patient Y, age 19, was hospitalized at Buckley Field Station Hospital from July 2 to July 28, 1943, with a characteristic initial attack of acute rheumatic fever. He was admitted to Lowry Field Station Hospital on July 29, 1943, with swelling, redness and tenderness of the knees, ankles and right shoulder and moderate precordial pain, increased by cough. No other abnormal physical findings were present. The temperature was 99° F. and the pulse rate was 96 per minute. The erythrocyte sedimentation rate was 1 mm. in 60 minutes. The Roentgen ray film of the chest showed normal lung fields and prominence of the left cardiac border. The electrocardiogram on July 30 showed inversion of P₂ and P₃.

The course continued unchanged for 2 weeks with an occasional elevation of temperature from 99.2° F. to a maximum of 100.2° F., slight tachycardia and joint pains, but repeatedly normal leukocyte counts and erythrocyte sedimentation rates.

The temperature remained normal from mid-August through the 1st week of September, there was improvement in the signs of joint involvement and the electrocardiogram showed upright P₂ and P₃ on August 30. A slight increase of joint activity and mild fever occurred in early September and on September 14, P₂ again became inverted. The electrocardiogram on October 9 showed

some inversion of P₂ and sharp inversion of P₃ as on admission to the hospital, although no definite clinical change was noted. On October 23, although a progressive improvement was noted clinically, the inversion of P₂ and flattening of P₃ persisted.

These waves became upright on October 27 but again showed deep inversion on Octo-

This case illustrates fluctuating changes in the P waves in Leads II and III, at times varying with the obvious clinical course, and at others alternating direction rather abruptly during a recovery phase, without accompanying signs of increased joint activity. In this patient there was likewise poor confirmation of the obviously active arthritis

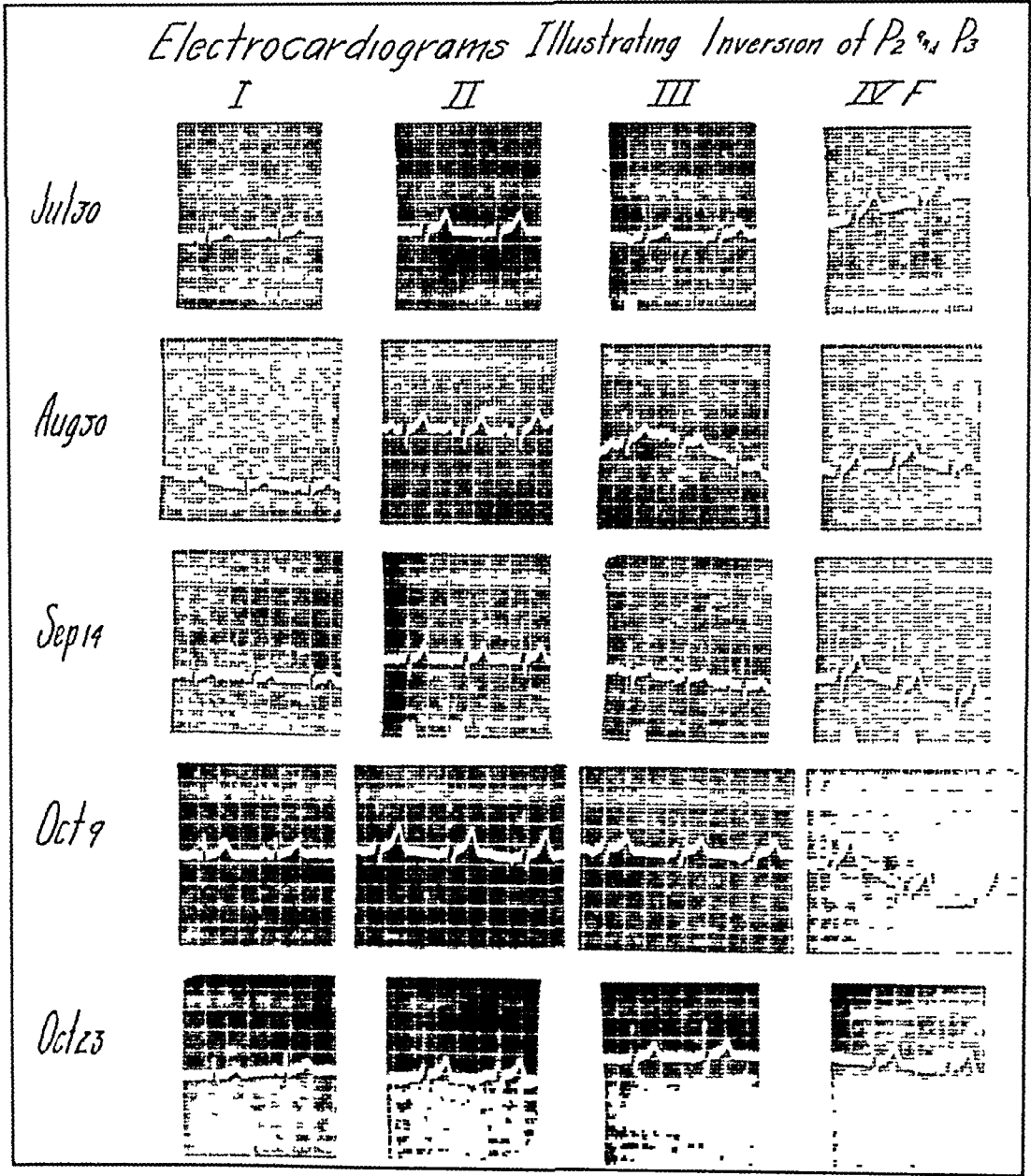


FIG. 1.—Case 2. Patient Y

ber 30. In early November clinical improvement was appreciable and the patient was first allowed to sit up on November 15. The electrocardiogram of November 18 showed a further return to upright of P₂ and P₃. Successive records showed no alteration during convalescence.

by consistent fever, increased leukocyte count or prolonged erythrocyte sedimentation rate. No significant electrocardiographic changes occurred other than in the P waves.

CASE 3. (Fig. 3.) Patient Z, age 20, was admitted to Lowry Field Station Hospital on July 23, 1943, having had a moderately

severe sore throat 3 weeks previously. Two days prior to admission he developed painful, reddened, swollen ankles. On admission his temperature was 99.2°F ., pulse rate 102 per minute, leukocyte count 9400 and an erythrocytic sedimentation rate of 25 mm. per hour (Cutler technique). The electro-

erythrocytic sedimentation rate fell to 14 mm. per hour on September 22 and to 4 mm. per hour on October 6. The electrocardiogram on September 2 and 21 showed upright P_1 and flattened P_{4r} .

There was obvious chronic tonsillar infection and 2 abscessed teeth. The latter

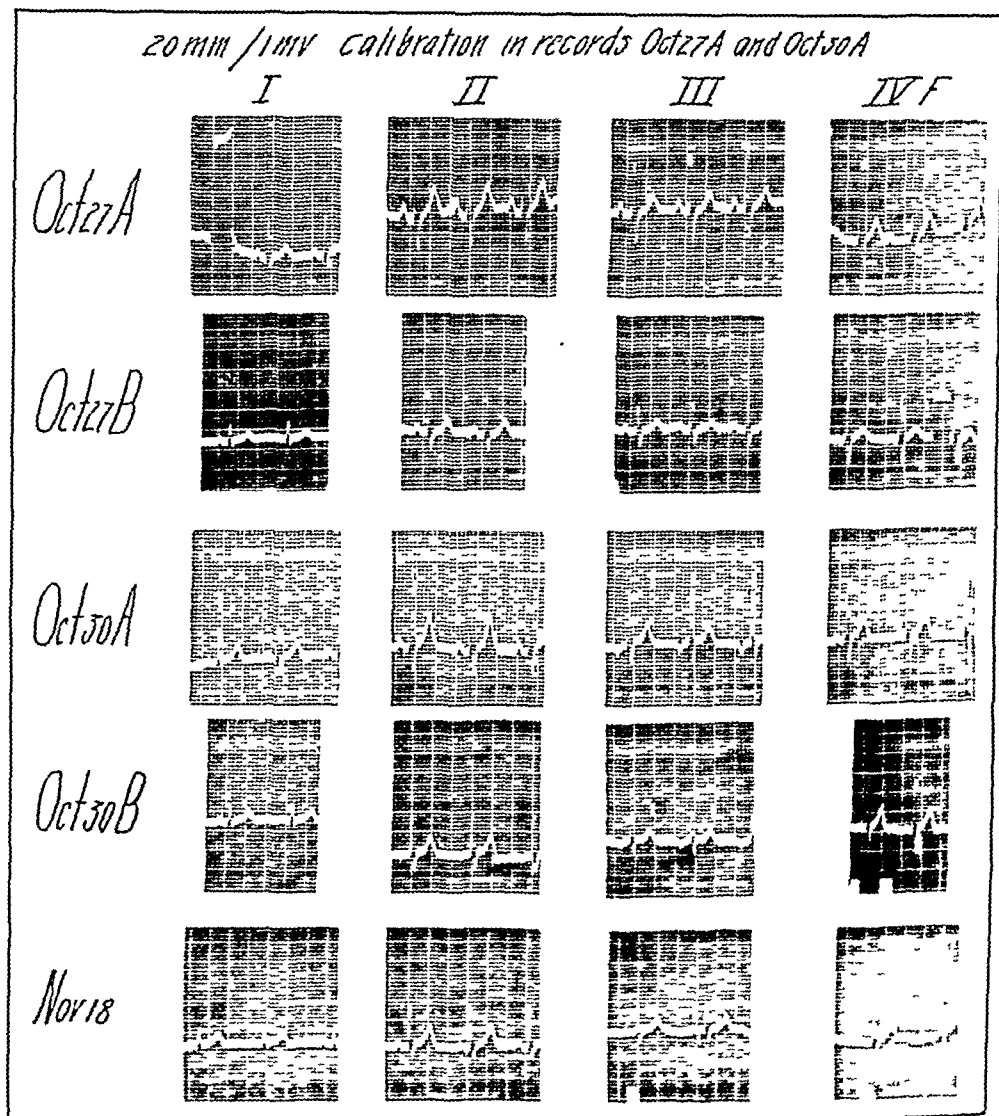


FIG 2.—Case 2. Patient Y.

cardiogram on July 28 showed inversion of P_1 and P_{4r} . Salicylate therapy was instituted and the joint involvement slowly subsided within the next month but the erythrocytic sedimentation rate was still 25 mm. per hour on August 16. The electrocardiogram was unchanged on August 26, with persistent inversion of P_1 and P_{4r} . The

were removed on October 9 and the former on October 25. He was transferred to the convalescent ward on November 3, where he remained free of joint symptoms until November 13. On that day he developed pain and swelling of the right knee and on November 18, of the right shoulder. His temperature varied from 100.2° to 101°F .,

and pulse rate from 96 to 104 per minute in those 5 days but both returned to normal by November 22 under resumption of salicylate therapy.

On November 17, his erythrocyte sedimentation rate was again 27 mm. per hour

monophasic inverted P wave at the 35 cm. and 40 cm. levels.

This case illustrated changes in P_i and P_{4r} occurring concomitantly with an initial acute rheumatic arthritis, subsiding and re-occurring with a relapse following tonsillec-

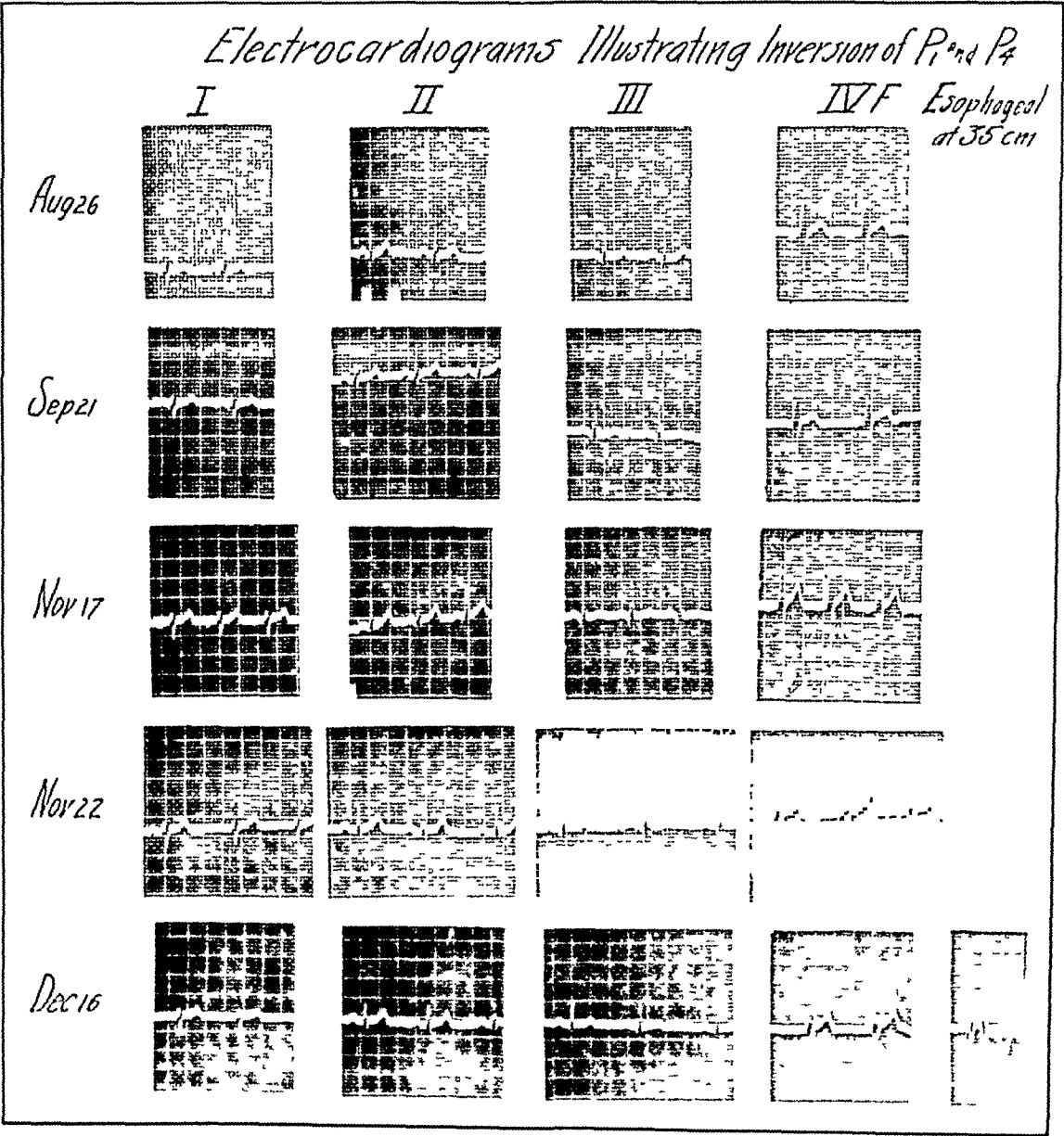


FIG. 3. Case 3 Patient Z.

and his leukocyte count was 15,200. The electrocardiogram demonstrated inversion of T_i and T₄. He gradually improved in the succeeding month, and on November 22 his electrocardiogram showed isoelectric P_r and upright P_i, the white blood count being 7600 on November 23. On December 16, the standard electrocardiograph leads were unchanged but the esophageal leads, taken by Lt. Col. Leonard Williams, showed a

tomy and extraction of teeth. The P wave changes receded to normal in this patient prior to the fall of the erythrocyte sedimentation rate to normal levels. No other significant deviations from the normal electrocardiogram were observed in this case.

No conclusive explanation can be offered for the development of these changes in the atrial electrocardiographic waves.

There have been minor differences of P-R conduction time observed in some of the records, but these differences were not sufficiently consistent to indicate a positive relation to the causal mechanism of the deviations of the P waves. Modification of the quality or direction of conduction through the muscle bundles of either or both auricles could account for the findings. This would notably be the case if it were generally accepted that such conduction takes place through certain bundle pathways and that inequality of contraction of the 2 auricles is not a rare phenomenon (Luisada⁶). It seems improbable that alteration of the pacemaker accounts for most of these progressive P wave changes,* although rarely an abrupt transient alteration of P wave form associated with variation in the P-R conduction time occurred, suggesting a shift of pacemaker.

Conclusions. 1. In 60 patients with acute rheumatic fever selected for detailed

study at the AAF Station Hospital at Lowry Field, Colorado, significant changes in the P waves of the electrocardiogram were observed in 8 of the patients and questionable alterations in 7 others.

2. These changes consisted of distinct flattening or inversion of the P waves and occurred most frequently in Lead III and Lead II, but were likewise observed, although less commonly, in Lead I and Lead IV F.

3. The alterations in the P waves generally paralleled the clinical evidences of active joint involvement and subsided with clinical recovery.

4. These P wave deviations are regarded as diagnostically important in that they often occurred unaccompanied by other electrocardiographic abnormalities and occasionally during periods when clinical signs of active rheumatic fever were associated with normal temperature, leukocyte count and erythrocyte sedimentation rate.

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* Since submission of this article for publication a comprehensive review has been published by Filshie, M. B., Griffith, A. C., Solley, R. I., and Leake, W. H. "Electrocardiographic Abnormalities in 6000 Cases of Rheumatic Fever," *Calif. and Western Med.*, 64, 340, 1946. These authors report that 1.63% of patients demonstrating P wave changes generally in Leads I and II. They favor a shift of pacemaker to account for these changes but do not correlate change in P-R interval with changes in form of P waves.

EVIDENCE OF MATERNAL RH SENSITIZATION WITHOUT EVIDENCE OF HEMOLYTIC DISEASE IN THE NEWBORN

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It has been repeatedly stated^{2,3,4,8} that hemolytic disease of the newborn can occur without the presence of demonstrable Rh antibodies in the maternal serum. The converse of this statement has, however, appeared to our knowledge only twice in the literature up to the present time. Dockera⁵ and Sachs⁶ reported 4 cases in which they were able to demonstrate what appeared to be Rh iso-agglutinins in the Rh negative mother's sera, yet these same mothers delivered 4 apparently normal Rh positive infants. The second report of this nature, was by Goldbloom and Lubinski,⁷ who record 1 example of Rh iso-immunization in the mother without symptoms of hemolytic disease in the infant.

The purpose of this paper is to describe 2 additional cases in which we were able to demonstrate Rh iso-antibodies in the maternal sera of Rh negative mothers without finding evidence of hemolytic disease of the newborn in their Rh positive offspring, and to discuss 2 mechanisms by which such apparent paradoxes could occur.

Case Reports. CASE 1. Mrs. T. is a 31 year old para I, gravida II, Wassermann

reaction negative. Her first pregnancy terminated in a criminal abortion at about 2 months of pregnancy, 5 years ago. The second pregnancy was entirely uneventful and terminated by the mid-forceps delivery of a living 3980 gm. male infant on Oct. 9, 1944. The infant appeared to be perfectly normal at the time of birth. The placenta weighed 690 gm., and was normal in appearance. On October 18, we received a sample of the mother's blood for Rh determination. This blood was brought to the laboratory merely because it was hospital routine to do Rh determinations on all obstetrical patients, not because of any clinical indication for the determination. At this time we found the mother to be Group A Rh negative and to have Rh agglutination antibody in her serum (Table 1). A second sample of the patient's blood obtained 2 days later, for checking, confirmed the findings on the original sample. Titration of the antibody in the second sample showed an anti-Rh titer of 1:4096. Incidentally, this is the highest titer we have observed in doing more than 2000 determinations for anti-Rh agglutinins.

At the time the antibody was found in the mother's serum, the infant was 10 days old. No blood studies had been carried out on the baby, but there had been no indication for these. In checking back with the pediatrician in charge of the case, as well as the

TABLE 1.—BLOOD GROUPS AND ANTIBODIES OF THE T FAMILY

	Date	Group	Rh	Maternal Rh antibody titer in saline*	Maternal Rh antibody titer in serum†
Mr. T.	3/10/45	A	Rh _i		
Baby T.	10/18/44	A	Rh _i		
Mrs. T.	10/18/44	A	Neg.	1:4096	
	3/10/45			1:32	
	9/21/45			1:32	
	1/19/46			1:32	1:256

* Maternal serum added to 2% suspensions of Rh positive test cells suspended in saline. Incubation. Read for agglutination.

† Maternal serum added to 2% suspensions of Rh positive test cells suspended in compatible serum. Incubation. Read for "conglutination."

obstetrician and interne, nothing abnormal was noted concerning the baby at birth nor during the first 10 days of its life. The only positive finding was a note that the baby was slightly icteric on October 14, the 6th day of life. This icterus had faded by the next day, and was interpreted as physiologic jaundice. The baby was discharged home on its 12th day of life at which time examination was entirely normal. The weight of the infant at the time of discharge was 4112 gm., a gain above birth weight of 130 gm. The infant was seen at frequent intervals from that time until it was over 18 months of age, and seemed to be normal at all times.

Typing of the infant's blood revealed it to be Group A Rh positive, and of the father's blood revealed him to be Group A Rh positive.* The titer of Rh agglutinin in the mother's serum has fallen since delivery, as shown in Table 1.

Admittedly the infant might have had subclinical hemolytic disease of the newborn, if such an entity exists. But, if one believes that disease is a definite morbid process having a characteristic train of signs and symptoms, then this infant was normal. It exhibited no edema, pallor, hepatomegaly, splenomegaly, bleeding tendencies, abnormal weight loss, nor feeding difficulty, but rather appeared to be a perfectly healthy baby in every way. However, it must be admitted that no blood studies were done on the infant, and also that icterus was present, but not until the 6th day of life, and lasted only about 24 hours. Certainly this would not be universally accepted as a sign of hemolytic disease of the newborn. The placenta was normal, and the ratio of its weight to that of the baby was normal, *i. e.*, 1:6.

The reason for the extremely high agglutinin titer is not clear. The patient had had 1 previous pregnancy, but this had been criminally interrupted at 2 months and hence it is unlikely that the mother received any great amount of sensitization to the Rh factor through that pregnancy. She has

never received blood intravenously nor intramuscularly. It is conceivable that the titer was not as high during the pregnancy as we found it to be 10 days postpartum. Unger and Wiener⁹ believe that at the time of placental separation there is a sudden shower of fetal red cells into the maternal circulation and that this is a potent factor in sensitizing the Rh negative mother against the Rh factor. If this hypothesis is true, we have then at least a partial explanation for the high titer observed in this case.

It is of interest to note that this patient's serum contained agglutinins of both the variety detectable in a saline system as well as the variety demonstrable in a serum or albumin system.^{5,10} The latter variety was not tested for until over 1 year postpartum, but its presence at that time is certainly indicative of its presence at the time of delivery. It is believed⁵ that agglutinins of the variety demonstrable only in serum or albumin systems exert a much more serious effect on the infant than those of the simple variety. Yet this infant exhibited none of the characteristics of hemolytic disease of the newborn.

CASE 2. Mrs. M.† is a 22 year old para II, gravida III, with expected date of confinement Jan. 25, 1946, Wassermann reaction negative. Her first 2 pregnancies ended by the delivery of full-term normal infants. The patient had never been transfused. We first came in contact with the case on Dec. 4, 1945. At that time we found the patient to belong to Group A and to be Rh negative (Table 2). In addition, Rh antibody of the type demonstrable in serum or albumin systems was found in her serum in titer of 1:16. None of the simple variety of antibody was demonstrable. The rise in the former type of antibody titer is recorded in Table 2 along with the subsequent appearance of the simple variety of Rh antibody and other pertinent findings.

On Jan. 28, 1946, the patient delivered a normal full-term infant weighing 3670 gm. The placenta was normal grossly and microscopically.

* The blood samples on all 3 members of this family were subsequently referred to Dr. Louis K. Diamond for checking and he reports as follows: Mr. T., Group A Rh⁺ homozygous; Baby T., Group A Rh⁺ heterozygous; Mrs. T., Group A Rh⁻ negative. Specificity of Rh antibody from Mrs. T. is of Rh variety.

We are indebted to Dr. Diamond for carrying out these tests.

† We are indebted to Dr. Floyd C. Bratt, Rochester, N. Y., for the opportunity of studying this patient, and to the Genesee Hospital, Rochester, N. Y., for making available the hospital records of this case.

The baby was observed carefully during the first 8 days of life and at no time was there any evidence of a hemolytic process present. Blood studies are shown in Table 3. There was no edema, pallor nor icterus. The infant took its feedings well, and was discharged home on its 8th day of life, weighing 200 gm. below birth weight. Careful follow-up since that time has revealed normal progress.

Lubinski⁷ state that the amount of antibody which passes through the placenta from the mother's circulation into the blood stream of the fetus varies widely in different cases, but they offer no explanation for this variation. They also believe that unknown factors in the fetus which they call "disposition" may be of importance.

TABLE 2.—BLOOD GROUPS AND ANTIBODIES OF MR. AND MRS. M.

	Date	Group	Rh	Maternal Rh antibody titer in saline*	Maternal Rh antibody titer in serum†
Mr. M.	1/10/46	O	Pos.	..	1:16
	12/ 4/45	A	Neg.	..	1:16
Mrs. M.	1/10/46	1:1	1:32
	1/22/46	1:4	1:32
	1/28/46	1:16
	3/13/46
Pregnancies					
No.	Year				
1	1943	3/13/46	O	Pos.	
2	1945	3/13/46	O	Neg.	
3	1946	1/28/46	O	Pos.	

* Maternal serum added to 2% suspensions of Rh positive test cells suspended in saline. Incubated Read for agglutination.
 † Maternal serum added to 2% suspensions of Rh positive test cells suspended in compatible serum. Incubated. Read for "conglutination."

TABLE 3.—BLOOD STUDIES ON BABY M (CASE 2)

Date	RBC (mill. per c.mm.)	Hb. (gm. per 100 cc.)	Smear (normoblasts per 100 WBC)
1/28/46	6 28	17 6	2
1/29/46	8 12*	25 8	1
2/ 1/46	6 47	20 6	
2/ 5/46	6 10	16 7	

* High levels probably due to dehydration.

It should be noted that the maternal serum contained Rh agglutinin of the type demonstrable only in serum or albumin systems almost exclusively and it was not until about 1 week before delivery that any of the simple variety was found.

Discussion. The birth of normal Rh positive infants from Rh negative mothers who have been sensitized to the Rh factor in the red cells of their fetuses can probably not be explained by our present knowledge of the forces involved. Dock-eray and Sachs⁶ state that perhaps the Rh antibodies pass the placental barrier only in certain conditions changing during the course of pregnancy. Goldbloom and

There occurs to us 2 other possibilities:
 1. Witebsky¹² has studied the occurrence of Rh substances in amniotic fluid and concludes that certain individuals are secretors of Rh and certain others are non-secretors. He bases this conclusion on the presence or absence of the Rh substances in amniotic fluids. He reports 3 cases of hemolytic disease of the newborn in which the mothers were Rh negative, the infants Rh positive and were non-secretors of the Rh factor. Boorman and Dodd¹ have found the Rh substance in tissue cells as distinct from the red blood cell.
 Maternal and fetal incompatibility of the major blood groups is of very frequent

occurrence, yet hemolytic disease as a result of this incompatibility is quite infrequent. The most logical explanation for this infrequency is that the vast majority of individuals ($\approx 80\%$) are secretors of the A or B factors and hence the antibody which the fetus obtains from its mother is to a large degree neutralized by the bulk of the body tissue, and only a relatively small amount is left free to react on the fetal red cells.

If this same logic is applied to the cases under discussion, we might say that these babies were secretors of the Rh factor, and hence the Rh antibodies which reached them by way of the placenta were largely taken up by the bulk of fetal tissue, and such a small amount remained to react with the red blood cells, that they were not sufficiently damaged to result in hemolytic disease. Unfortunately, no attempt could be made to study the amniotic fluid in either of the cases presented.

2. Wiener¹¹ in describing his "conglutination" test states that it occurs in 2 stages, and that a third component besides the Rh antigen and the specific antibody is necessary for the reaction of clumping to occur. He describes this third component as a colloidal substance in the plasma, possibly identical with the so-called X-protein, and calls it "conglutinin." He believes that in those infants which do not present signs of hemolysis for several hours or even days after birth, the "conglutinin" does not form until after birth, and that when it finally does form, the infant then shows evidence of hemolytic disease.

By similar reasoning one might say that

in certain cases when normal Rh positive infants are born to Rh negative mothers whose serum contains Rh antibodies, those infants are normal because the particular antibody in the maternal serum cannot react with the antigen on the fetal red cells because of the absence of the third necessary component, "conglutinin." This could possibly be the explanation in the second case presented since only the "conglutinating" type of antibody was present in the maternal serum until the last week before delivery at which time a small amount of the simple variety of antibody was also found.

We believe that cases of the type described here and previously are of interest only to workers in the field. Obstetricians and pediatricians should continue in the belief that when Rh antibodies are found in the serum of a pregnant woman, that woman will probably deliver an infant with hemolytic disease. Believing this, he should do everything possible to lessen the severity of the disease in the newborn, i. e., induction of labor when indicated, followed after birth by immediate and repeated transfusions of Rh negative blood to the infant, or by very close observation, including red blood cell counts and hemoglobin determinations twice daily for the first 5 or 6 days of life, then daily for the next week. These babies, of course, should not receive the mother's milk.

Summary. 1. Two cases are presented in which apparently normal Rh positive infants were born to Rh negative mothers whose sera contained Rh antibodies.

2. Two possibilities to explain these unusual cases are discussed.

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RESPONSE TO PENICILLIN BY A TYPHOID CARRIER

STUDIES ON PENICILLIN SENSITIVITY OF STRAINS OF TYPHOID BACILLI

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PENICILLIN now has become more readily available and recently it has been possible to treat 2 typhoid carriers with fairly large doses of the substance. It is believed that 1 of the patients, found to be a very definite gall bladder carrier, was apparently cured as the result of treatment while the other, whose focus of infection was not as definite, received only a temporary effect. In an effort to explain this apparent cure with penicillin, a collection of various strains of *Eberthella typhosa*, including that of the successfully treated carrier, was analyzed for *in vitro* susceptibility to penicillin. It was possible to demonstrate some differences between the organism of the patient and those from other sources. Also, it is thought that the ability of the liver to concentrate penicillin was responsible in part for the good result achieved in the patient.

The history and treatment of the patient displaying a good response is as follows:

Case Report. CASE 1. A. C., a Negro male, age 31, was discovered to be a typhoid carrier following a routine check by rectal swab of food handlers on a Navy base. On investigation, it was found that he had been apparently involved in an outbreak of typhoid fever in his home district of Louisiana in 1927 when he and members of his family were ill at the same time with a disease known as "typhoid malaria." He had an uneventful recovery, and believed himself entirely well thereafter. He held various jobs as a laborer and enlisted in the Navy as mess steward in January of 1944.

When discovered to be harboring *E. typhosa* he was working as a food handler in an officer's mess, this being his first work in handling food. No typhoid fever had been found in any Naval personnel of the base and the man had not been on leave. Rectal swab cultures on bismuth sulfite or S. S. agar taken at anytime showed almost pure cultures of typhoid bacilli, and bile cultures were also markedly positive, displaying pure growths of typhoid on each culture. Urine cultures were negative.

A Graham test demonstrated reduced function of the gall bladder, with at least 1 small calcified stone present but the patient reported no symptoms in the past suggestive of gall bladder disease. Because of the markedly positive bile cultures it was decided that his major focus of infection was in the gall bladder and operation was proposed. This was firmly refused by the patient.

During the 4 month period prior to the institution of penicillin therapy, various recommended therapies had been attempted without success. A course of sulfadiazine, consisting of 32 gm. in a 5 day period, was given the patient, but there was no apparent effect, rectal cultures remaining positive during and after treatment. A 5 day course of gall bladder dye was then administered, but this also showed no effect on his positive cultures.

When it was found by *in vitro* studies of the patient's organism that complete inhibition occurred on blood agar plates with penicillin levels of 8 to 9 Oxford units per cc. of blood agar, penicillin therapy was administered according to the following dosage:

While receiving penicillin, fluids were

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limited to 800 cc. daily and the patient was allowed to have the regular hospital diet.

After 2,350,000 units of penicillin had been administered over a 7 day period the patient refused to go on with treatment. He was returned to duty and observed but was not allowed to handle food. Since the termination of penicillin treatment the patient has been asymptomatic and has remained free of typhoid organisms. He has now shown negative rectal cultures for a period of 5 months. Cultures of bile were negative for typhoid 2 and 5 weeks after the end of treatment. Rectal swab cultures always had been markedly positive for *E. typhosa* during the 4 month period while he was under observation prior to receiving penicillin.

Rectal swab examinations were consistently positive for typhoid. A bile examination revealed only 4 to 5 colonies of typhoid per cc. of bile and there was some doubt as to whether or not he was a gall bladder carrier. Penicillin sensitivity tests were carried out with this strain as recorded in Tables 3 and 4, and he was later placed on the following régime of penicillin therapy as a trial.

Unfortunately, orders to limit the patient's fluids to 800 cc. daily were not carried out and the patient drank a normal intake each day.

After receiving 3,450,000 units of penicillin over a 9 day period this patient showed negative cultures temporarily (i. e., for a period of 1 week) but cultures later became

TABLE 1.—INHIBITION OF GROWTH BY PENICILLIN (CASE 1)

Day	Date	Total daily dose (Oxford units*)	Cultures		
			Rectal swab	Bile	Urine
	12/ 7, 44—	...	8 Pos.	2 Pos.	1 Neg.
1	4/11/45	200,000			
2	4/12/45	400,000			
3	4/13/45	400,000	Less than 5 col- onies per plate		
4	4/14/45	400,000	"		
5	4/15/45	400,000	"		
6	4/16/45	400,000	Neg.		
7	4/17/45	150,000	"		
	Total:	2,350,000			
13	4/23/45	.	"		
17	4/27/45	..	"		
21	5/ 4/45	.	..	Neg.	Neg.
35	5 15 45	.	Neg.		
49	5, 29 45	.	"	Neg.	
55	6/ 4 45	.	"	..	Neg.
101	7 20 45	.	"		
137	8 25 45	.	"		

* 50,000 units intramuscularly every 3 hours.

CASE 2. This case reported having had typhoid fever 3 years ago. This man, H. B., a 25 year old German prisoner of war was first discovered to be a typhoid carrier during an outbreak of typhoid fever in a prisoner of war camp. After an investigation had been made, it was concluded that he was responsible for 3 cases of typhoid fever in his fellow prisoners, with 1 of the case ending fatally. The original infection of the carrier was contracted while he was fighting on the German Front in Russia. Since then he had believed himself well and, before the present outbreak, had noted no cases of typhoid in his vicinity.

positive and have remained so. Since it was necessary to transfer the patient to another hospital, he was no longer available for a re-trial with penicillin. Further attempts at therapy using a longer period of administration of larger doses of penicillin were felt to be probably justified, because of the temporary negative cultures found.

The possibility was considered that strain variation in susceptibility of *E. typhosa* might exist and that, if such were found, a possible explanation would be available for the apparent cure of 1 of the carriers. *In vitro* studies were made on a

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group of various strains of typhoid organisms.

Eight different strains of *E. typhosa* were obtained and simultaneous evaluation made for the effect of varying concentrations of penicillin upon 24 hour broth cultures of the different organisms. None of the patients furnishing the strains had ever received penicillin.

organism from a urinary carrier; E: laboratory strain; F: laboratory strain.)

There is a definite difference noted in strain susceptibility to penicillin, especially when the higher concentrations of penicillin are added to broth cultures, but it is presently unknown whether or not these inhibitory concentrations of penicillin ever could be attained in a human gall bladder

TABLE 2.—INHIBITION OF BACTERIAL GROWTH BY PENICILLIN (CASE 2)

Day	Date	Total daily dose (Oxford units*)	Cultures	
			Rectal swab 2 Pos.	Bile Pos. Urine Pos.
0	8/14/45	250,000	Pos.	Pos.
1	8/24/45	400,000	"	
2	8/25/45	400,000	"	Neg
3	8/26/45	400,000	Pos.	
4	8/27/45	400,000	Neg.	"
5	8/28/45	400,000	"	
6	8/29/45	400,000	"	"
7	8/30/45	400,000	"	
8	8/31/45	400,000	"	"
9	9/ 1/45	3,450,000	"	
	Total:		Pos.	
10	9/ 2/45		"	
13	9/ 5/45		"	
20	9/12/45			
21	9/13/45			
22	9/14/45			

* 50,000 units intramuscularly every 3 hours.

TABLE 3.—DEGREE OF GROWTH* OF TYPHOID BACILLI IN BROTH CONTAINING VARYING AMOUNTS OF PENICILLIN

Oxford units of penicillin per cc. of broth	A. C.†	H. B.†	A. G.	T. B.	C.	D.	E.	F.
0	4	4	4	4	4	4	4	4
5	4	4	4	4	3	4	3	4
10	3	3	3	3	2	4	2	4
15	2	1	2	1	1	3	1	2
25	2	1	2	1	1	3	1	1
30	1	1	1	1	1	2	1	1
50								

* Degree of growth in broth graded 1 to 4.

† Organisms from carrier successfully treated.

‡ Organisms from carrier showing only temporary response.

(Origin of typhoid strains: A. C.: phagetype "A" strain from typhoid carrier who, under treatment, made a favorable response to penicillin; H. B.: strain from German P. O. W. carrier unsuccessfully treated with penicillin; T. B.: strain from mild case of typhoid fever in German P. O. W. outbreak; A. G.: strain from German P. O. W. dying of typhoid fever; C: phagetype "C" from carrier; D: or-

carrying typhoid organisms. It is of interest, however, that while one gall bladder carrier, A. C., received apparent beneficial effect from penicillin, his organism by this type of *in vitro* examination displayed no startling difference from that of the resistant carrier, H. B.

The incubation of tubes of broth and penicillin that have been inoculated with typhoid organisms clearly does not repre-

sent *in vivo* methods; much, therefore, remains conjecture. A different method of demonstrating strain variation consisted of inoculating a loopful of a 24 hour broth culture of a particular strain upon the surface of a blood agar* plate containing various amounts of penicillin.

far above that obtained therapeutically might be needed for microscopic inhibition of broth cultures of typhoid, smaller concentrations of penicillin would still cause microscopic changes in the appearance of the organisms, even though the organism survived. Exposure of broth

TABLE 4.—GROWTH* OF STRAINS OF TYPHOID BACILLI ON BLOOD AGAR PLATES CONTAINING VARYING AMOUNTS OF PENICILLIN

Oxford units per cc. of blood agar	A. C.†	H. B.‡	A. G.	T. B.	D.	E.	F.
4 1	2	1	3	..	4	1	
5 0	2	3	4	1	4	1	3
5.3	1	4	1	0	4	0	
6 8	1	2	1	2	4	0	2
7.6	1	1	1	1	1	0	1
8.4	1	0	1	1	1	0	
9.0	0	0	0	0	0	0	
9.8	0	0	0	0	0	0	

* Degree of growth graded from 1 to 4.

† Organism from carrier successfully treated.

‡ Organism from carrier showing only temporary response.

In this series it is of interest to note that much smaller amounts of penicillin, when in a blood plate, cause total inhibition of growth. However, the same relative difference in strain susceptibility was again noted. The "D" strain proved the most resistant and the "E" strain the most sensitive. The "A. C." strain, from the carrier successfully treated with penicillin in both series, demonstrated a sensitivity in the middle ranges, but when on a blood plate it appeared somewhat more sensitive than the organism of the carrier H. B., the latter displaying only a temporary response to treatment.

Comment. It has been stated^{2,3,5} that penicillin is ineffective in the treatment of typhoid fever and other infections with gram negative bacilli, but in the asymptomatic and persistent carriers of typhoid organisms, and especially in those individuals who possess a focus of typhoid infection in the gall bladder, factors may be present which allow a response to the effect of penicillin.

Abraham and Florey¹ in early work with penicillin reported an interesting effect on typhoid and other organisms. They found that, though a concentration of penicillin

cultures to dilutions of penicillin containing a concentration one-sixth that of the total inhibiting dose would bring about definite microscopic changes in structure and it was intimated that possible therapeutic effects might be obtained by these same high dilutions of penicillin. Hobby⁴ was able to demonstrate a relative sensitivity of typhoid organisms to penicillin and Thomas³ observed unusual structural forms of gram negative bacilli after exposure to dilutions of penicillin greater than those needed for complete bacteriostasis. Steiner⁷ also described considerable variation in sensitivity to penicillin of various gram negative bacilli.

The findings by Abraham and Florey¹ that higher concentrations of penicillin occurred in cat's bile than in the blood serum of the animal, suggested that a beneficial effect might be obtained in treating gall bladder typhoid carriers with penicillin. Rammelkamp⁶ was able to show that penicillin was present in human bile in higher concentrations than in blood serum and concluded that penicillin is perhaps concentrated by the liver. Such a concentrating power might possibly allow effective penicillin levels to be

* Nutrient agar plus 10% by volume of defibrinated sheep's blood made up as a pour plate. Penicillin added prior to pouring plate.

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reached in treating gall bladder typhoid carriers.

Considerable work has appeared demonstrating variations in susceptibility to penicillin of strains of staphylococci and other organisms. The susceptibility of certain strains of the typhoid bacillus as demonstrated above could be a third factor for the successful treatment of a typhoid carrier, especially one whose infection is primarily in the gall bladder. However, it is presently unknown whether or not the inhibitory concentrations of penicillin required for susceptible strains as demonstrated by *in vitro* methods could ever be attained in a human gall bladder.

Still another factor that might be responsible for a penicillin effect on typhoid organisms would be the presence in certain lots of ordinary commercial penicillin of unusual amounts of substances of the streptomycin type, or related groups. The penicillin used in treating the carrier "A. C." was manufactured by the Cutter Company and was produced by the shallow flask method.

It is admittedly very difficult to deter-

mine when a typhoid carrier is cured, but in the patient A. C. mentioned above, there has been an apparent response to penicillin therapy which has persisted for 5 months. Carriers have relapsed after such an interval but the response made, even if in only 1 patient, justifies further trials with similar therapies on larger groups of patients.

Summary and Conclusions. 1. A typhoid carrier of the gall bladder type made a response to large doses of penicillin and has remained free of typhoid bacilli for 5 months.

2. A second typhoid carrier similarly treated with penicillin made only a temporary response.

3. Among the strains of typhoid organisms tested, strain variation in susceptibility to penicillin was found to occur and this variation was not the result of previous treatment with penicillin.

4. Large doses of penicillin may be considered to be of value in the treatment of the typhoid carrier, but possibly only in the gall bladder type of carrier who has an organism sensitive to penicillin.

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BACTERIOLOGIC, CLINICAL AND PATHOLOGIC EXPERIENCE WITH 86 SPORADIC CASES OF SALMONELLA INFECTION*

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At the Queens General Hospital, 86 cases of *Salmonella* infection have been found since 1941, from which 21 different species of *Salmonella* have been isolated. The distribution, the type of syndrome, the severity and the sources of the various species are indicated in Table 1. The species with the highest incidence, *S. typhimurium*, *S. cholerae suis*, and *S. oranienberg*, accounting for 11, 20 and 16 cases respectively, showed interesting differences as to source, severity of symptoms, the absence of symptoms, or the development of the carrier state. There were 13 deaths, with 9 autopsies. Of the 86 cases, 19 had gastro-intestinal symptoms, 9 had general symptoms only, and 27 had both gastro-intestinal and general toxic manifestations. In addition to the 13 patients who died, 22 patients were severely ill and almost an equal number, (21) had only mild symptoms. The microorganisms were recovered from stools in 70 instances, from the blood in 11, and from the urine in 3, the others representing double infections.

Method and Material. The method for isolating enteric pathogens has been in use in this laboratory since 1941, and has been described by Mollov *et al.*⁵ Inoculations were made simultaneously on one plate of S.S. agar, on plated MacConky media and in one tube of tetrathionate broth. Growth from this last was subcultured on MacConky agar only. From each plate, 2 to 4 of each type of non-lactose fermenting colonies were subcultured to Krumwiede triple sugar slants. One positive or suspicious slant from each case was studied for detailed fermentation reactions. All *Shigella* species were identified serologically and checked by

the New York City Department of Health. After biochemical fermentation determinations, the *Salmonella* were identified serologically by the Beth Israel Hospital *Salmonella* Typing Center.

A total of 3206 stools were examined; 752 from hospitalized patients with intestinal disorders, and 2454 specimens from dietary workers, nurses and interns, all apparently in good health.

During this 4 year period, typhoid bacilli were isolated 19 times; 16 from patients ill with the disease and 3 from carriers (2 convalescent—1 for 30 years; and 1 a healthy carrier).

In most of the individuals from whom a species of *Salmonella* was isolated, more than 1 positive stool was obtained, the average being 3 positives. Of the 86 individuals harboring *Salmonella* microorganisms, 55 were hospital patients (27 females and 29 males), and 30 were from the surveyed dietary workers, nurses and interns. Of these, only 2 were males while 28 were females, because of the predominance of female nurses in this latter group.

Stools were studied routinely while blood and urine cultures were made only when particular indications existed.

Cultural and Clinical Findings. A wide range of *Salmonella* species was implicated. In general, the gastro-intestinal syndrome was most frequent, and the microorganisms were found most often in the feces. The variations in the syndromes and in their severity were remarkable when the infections were due to different species, but were also quite marked when different cases of infection with the same species were compared. The clinical variations were also found in instances when several

* Presented before the New York State Association of Public Health Laboratories in Albany, New York, on Nov. 2, 1945.

simultaneous infections in several members of a family with the same organism occurred. Only 3 species, *S. typhi murium*, *S. cholerae suis* and *S. oranienberg*, offer sufficient instances for any generalizations whatsoever.

terium was recovered from 1 stool only. In interpreting the cultural findings, it must be realized that routine blood and urine cultures were not taken in most cases.

In general, there is a tendency for cer-

TABLE 1.—CLASSIFIED CLINICAL AND PATHOLOGIC DATA IN SPORADIC SALMONELLA INFECTIONS

	Total cases	Syndrome			Severity				Source of culture		
		G.I.	General	Both	Carrier	Mild	Severe	Death	Blood	Feces	Urine
<i>S. typhi</i> (<i>E. typhosa</i>)	19	1	9	6	3	2	13	1	11	19	1
<i>S. cholerae suis</i> with var. Kunzendorf 2	11	0	6	5	0	0	7	4	8	1	2
<i>S. typhi murium</i>	20	3	3	9	5	5	5	5	2	19	1
<i>S. oranienberg</i>	16	3	0	2	8	3	2	0	1	12	0
<i>S. derby</i>	5	0	0	0	5	0	0	0	0	5	0
<i>S. newport</i>	5	1	0	2	1	3	0	1	0	4	0
<i>S. urbana</i>	2	2	0	0	0	1	0	1	0	2	0
<i>S. schottmulleri</i> (Para B)	2	0	0	2	0	0	2	0	0	2	0
<i>S. give</i>	2	1	0	1	0	0	2	0	0	2	0
<i>S. montevideo</i>	3	0	0	1	2	0	0	1	0	3	0
<i>S. barcelly</i>	1	1	0	0	0	1	0	0	0	1	0
<i>S. london</i>	1	1	0	0	0	1	0	0	0	1	0
<i>S. thompson</i>	1	0	0	1	0	0	1	0	0	1	0
<i>S. oregon</i>	1	0	0	1	0	0	1	0	0	1	0
<i>S. panama</i>	4	1	0	2	1	1	2	0	0	4	0
<i>S. anatum</i>	1	0	0	0	1	0	0	0	0	1	0
<i>S. lichtfeld</i>	1	0	0	1	0	0	0	1	0	1	0
<i>S. moribificans bovis</i>	6	4	0	0	2	4	0	0	0	6	0
<i>S. muenchen</i>	1	1	0	0	0	1	0	0	0	1	0
<i>S. manhattan</i>	1	0	0	0	1	0	0	0	0	1	0
<i>S. newington</i>	1	0	0	0	1	0	0	0	0	1	0
<i>S. minnesota</i>	1	1	0	0	0	1	0	0	0	1	0
Totals*	86	19	9	27	27	21	22	13	11	70	3

* Exclusive of *S. typhi*.

S. oranienberg presented the mildest syndromes, with 11 carriers giving no symptoms at all, and 3 presenting a mild picture with gastro-intestinal symptoms only. Two cases of *S. oranienberg* infection did present some toxic general manifestations, but there were no deaths. Most of the severest illnesses were found in cases of *S. cholerae suis*. All 11 of these cases showed toxic general manifestations, 5 with gastro-intestinal symptoms. There were 4 deaths, and the remaining cases were severe in this group. There were 8 cases of positive blood culture with the latter microorganism, while only 1 positive culture was obtained from the blood with the *S. oranienberg* infections. This microorganism was isolated in 12 instances from the stool, in contrast to the cases of *S. cholerae suis* infection, where the bac-

terium was recovered from 1 stool only. In interpreting the cultural findings, it must be realized that routine blood and urine cultures were not taken in most cases. In general, there is a tendency for cer-

tain species to produce a more or less characteristic and typical syndrome. Allowing for some exceptions and variations, *S. cholerae suis* seems to cause the picture of sepsis with greatest uniformity, with and without "seeding foci" (Bornstein³). *S. oranienberg* shows a definite tendency to produce a milder clinical picture, or absence of all symptomatology in the form of the "carrier state." The gastro-intestinal picture with diarrhea or vomiting is the usual form that symptomatic infection with this organism assumes (Seligman, Saphra and Wassermann;¹⁷ Sachs and Antine;¹⁸ Jager and Lamb⁴).

S. typhi murium occupies a position in between *S. cholerae suis* and *S. oranienberg* in all of its manifestations and in its severity. In the 20 instances of *S. typhi murium* infection, the observed features of

the illness suggested an intermediary position from the standpoint of severity, the tendency to intestinal symptoms, and the tendency to invade the blood stream or to remain within the intestinal content. Thus, 5 of the 20 cases were asymptomatic carriers, only 3 presented general septic symptoms alone, while 9 showed gastrointestinal manifestations with accompanying general toxic symptoms. The micro-organism was recovered from the feces in 19 cases, from the blood in 2 cases, and once from the urine.

Pathology. Nine of the 13 patients who died came to autopsy, representing the largest group as yet reported. The post-mortem data will be found in Table 2. One death, not coming to autopsy, was a Medical Examiner's problem in a 2½ year old child, admitted for extensive burns involving 63% of the body, who developed diarrhea after 3 weeks in the hospital,

while apparently doing well. Blood culture yielded *S. typhi murium*. The patient developed a high temperature with convulsions, and died within a few days. This case may represent the activation of a latent carrier state or an infection acquired while on the ward, though a careful survey for potential carrier sources among the hospital personnel was unavailing. Of the 3 remaining patients who died and were not examined at autopsy, 1 had clinical pyelonephritis with *S. cholerae suis* in the urine; a second was an infant with congenital heart disease who developed an upper respiratory infection and diarrhea with *S. urbana* in the stool; and the last was a case of Wernicke's syndrome with advanced cirrhosis of the liver, in whom the infection may well have been a terminal blood stream invasion. The data from these unautopsied cases are given in Table 4.

TABLE 2.—AUTOPSY FINDINGS IN DEATHS WITH POSITIVE SALMONELLA CULTURES AT QUEENS GENERAL HOSPITAL

Case No.	Age	Culture	Source	Syndrome	Pathology and diagnosis
1—A-42-242	21	<i>S. typhi murium</i>	Stool, ulcers, peritoneum	G.I., acute appendicitis, operated	Typhoid-like ileocolitis with hyperplastic Peyers patches
2—A-41-489	33	<i>S. typhi murium</i>	Stool, blood, spleen, thigh, throat	G.I., diarrhea, chill with shock	Sepsis, with cellulitis of thigh; hyperplastic mesenteric nodes
3—A-45-218	18 mos.	<i>S. typhi murium</i>	Blood, lung, stool	Diarrhea, then convulsions, coma, high temperature	Hyperplastic Peyer's patches mesenteric nodes, bacteremia
4—A-44-49	68	<i>S. cholerae suis</i>	Kidney pus and blood	Cardiac and cerebral with coma (uremia?)	Suppurative pyelonephritis
5—A-43-477	9	<i>S. cholerae suis</i>	Blood	Cerebral meningeal and then encephalic	Chr. cholangitis with biliary cirrhosis; hyperplastic Peyer's patches
6—A-43-27	26	<i>S. cholerae suis</i>	Stool	G.I. diarrhea with bleeding	Chr. active ulcerative colitis
7—A-44-135	46	<i>S. tichfeld</i>	Stool, bile, stomach	G.I., typhoid-like	Marked gastritis and bronchopneumonia
8—A-44-226	60	<i>S. newport</i>	Pericardium	Chest pain—coma, diabetic	Suppurative pericarditis, cholangitis
9—A-41-455	1½ mos.	<i>S. monterideo</i>	Stool	G.I.—diarrhea	G.I. tract negative; bronchopneumonia and prematurity

TABLE 3.—AUTOPSY FINDINGS IN *S. TYPHI MURIUM* CASES ON FILE AT BETH ISRAEL SALMONELLA TYPING STATION

Case No.	Age Sex	Source	Syndrome	Pathology
1	74 M	Spleen, bone	G.I., diarrhea, splenic tumor, severe anemia	Infected dissecting aneurysm of aorta with regional osteomyelitis, chronic cholangitis with cirrhosis
2	66 M	Stool	G.I., diarrhea	Acute arteritis, bronchopneumonia
3	66 M	Stool, from all path. sites	G.I.—severe diarrhea, vomiting, collapse	Pus filling gall bladder and in right ureter, petechiae in ileum and sigmoid
4	49 F	Urine, spleen and intestinal wall	G.I.—outbreak of food poisoning with vomiting and diarrhea, uremia	Pus in urine, kidneys enlarged
5	5 wks. F	Stool, blood	G.I.—diarrhea, vomiting	Thrombophlebitis with sepsis after intravenous
6	62 M	Blood and mitral valve	Fever only, no G.I.	Endocarditis
7	6 mos. M	Blood (Pn. IV), then <i>Typhi murium</i>	G.I.—otitis with meningitis, then diarrhea	Possibly pneumonia?
8	6 wks. F	Stool and blood	G.I.—diarrhea, pneumonia	No data
9	50 F	Blood and spleen	G.I.—vomiting and diarrhea	No data

In general, the lesions found in the patients who came to autopsy presented a localization in the gastro-intestinal tract in 5 cases, with 2 instances of chronic cholangitis in addition (Cases 5 and 8); localization in the kidney in 1 case (Case 4); and involvement of the respiratory tract in 2 cases (Cases 3 and 6). It will be noted that 3 *S. typhi murium* cases came to autopsy, with a septic picture in 2 of them and a typhoid-like picture in 1 (Case 1, Figs. 1, 2, 3 and 4). In none of these 3 cases did there exist independent, obviously preëxisting pathologic lesions of sufficient magnitude to be significant in the mechanism of death. The Salmonella infections in the non-autopsied cases may well represent the insignificant incidental states, though the acute final episode suggests that the Salmonella infection was a lethal terminal event. Salmonella infection was the direct primary cause of death in all the cases coming to autopsy. This fact is significant of itself. In general, one is impressed by the mimicry of the different clinical forms and pathologic states of typhoid, and by the not infrequent severity of the infection.

again prominent. A further analytical discussion of this group would be repetitious. Only 2 of the cases showed general symptoms of sepsis only. The remaining 15 cases showed some gastro-intestinal symptoms along with severe general toxic manifestations. Others showed gastro-intestinal symptoms only, but this would hardly be expected often, since the group is a select one of cases severe unto death.

The first autopsy of this series (Table 2) was performed by Dr. N. Mitchell in 1941 on a patient who was operated upon for acute appendicitis and died of peritonitis. At autopsy, this patient showed the typical morphologic picture of typhoid fever (Figs. 1, 2, 3 and 4). The micro-organism cultured from several sites was *S. typhi murium*. A few months later, another patient was admitted with a clinical picture of typhoid fever, and from his blood stream *S. typhi murium* was also isolated. At autopsy, this second case failed to show the characteristic hyperplastic lesions in Peyer's patches, though the mesenteric lymph nodes were hyperplastic (Case 2, Fig. 5). In a third

TABLE 4.—CLINICAL DIAGNOSES IN SALMONELLA DEATHS WITHOUT AUTOPSY AT QUEENS GENERAL HOSPITAL

Case No.	Age	Culture	Source	Syndrome	Diagnosis
1—(L. W.)	48	<i>S. typhi murium</i>	Blood	Coma, peripheral neuritis	Cirrhosis of liver with Wernicke's syndrome
2—(M. P.)	2	<i>S. typhi murium</i>	Stool	Diarrhea; convulsions	Extensive first and second degree burns with diarrhea 3 wks. after admission
3—(K. G.)	69	<i>S. cholera suis</i>	Urine	Uremic and cardiac	Hypertensive heart disease; pyelonephritis (?)
4—(K. A.)	4	<i>S. urbana</i> mos.	Stool	Bronchitis with diarrhea	Congenital cardiac with cyanosis

We studied the clinical course of 17 *S. typhi murium* deaths encountered by the National Salmonella Center at Beth Israel Hospital. The records of this group of cases were generously made available by Dr. Seligmann. The available findings in the 9 cases studied at autopsy are given in Table 3. For the entire 17 cases, a total of 14 positive cultures were obtained from the blood, 5 from the feces, 3 from the spleen, 2 from the urine, 2 from the intestinal wall, and 1 each from the liver, gall bladder and mitral valve. The septic picture was

case, that of an 18 month old child who was thought to have encephalitis because of coma and convulsions, hyperplastic Peyer's patches and mesenteric nodes, without ulceration of the gastro-intestinal tract, were found. The other instance in which hyperplastic Peyer's patches were found at autopsy represented an infection with *S. cholera suis*. The patient was a 9 year old child with chronic cholangitis with biliary cirrhosis. In 1 case in which a clinical diagnosis of typhoid fever was made, there was only marked gastritis (Case 7, Fig. 6), with no changes in



FIG. 1.—Case 1. The cecum and small intestine showing hemorrhagic hyperplastic Peyer's patches and lymphoid tissue and superficial ulcers of ileum and cecum. (Approx. $\times \frac{1}{2}$.)



FIG. 2.—Case 1. Minute ulceration of large bowel showing fibrin and predominant mononuclear exudate. (Low power; approx. $\times 40$.)

Peyer's patches; *S. lightfield* was isolated. The remaining autopsies showed no morphologic alteration in the gastro-intestinal tract (Table 2).

Autopsy disclosed only 2 cases with hyperplasia of Peyer's patches (Cases 1 and 3), identical to that seen in typhoid fever. Study of the autopsied cases, however, emphasizes the frequency of blood stream invasion and sepsis in the fatal cases. In the few cases where a routine

blood culture was taken clinically in this group, the significant microorganisms were found. One case showed ulcerative colitis. In general, these, as well as other morphologic lesions which were found, all speak for bacteremia. The septic clinical picture associated with the typhoid-like syndrome encountered in Cases 1, 2, 8 and with the respiratory tract involvement in Cases 8, 10, 11 and 12, all favor an existing bacteremia, despite the ab-



FIG. 3.—Case 1. Region of Peyer's patch showing prominent mononuclear exudate and fibrin. (Medium power; approx. $\times 40$.)



FIG. 4.—Case 1. Higher magnification showing characteristic mononuclear exudate. (High power; approx. $\times 320$.)

sence of proof by positive blood cultures in many instances. Such bacteremias are known to occur quite regularly in cases of typhoid and pneumonia.

Most of the severe and nearly all of the fatal cases presented a septic picture with bacteremia as shown by blood culture. The fatal cases in which blood stream invasion was not demonstrable represented sudden deaths with inadequate study, young children or newborn nursery

victims. The tendency to belittle *Salmonella* infections is not justified by the clinical course often seen in the very young. In Case 8, there was a localization of such a blood stream infection in the pericardium (Fig. 7), and a cholangitis, with *S. newport* recovered from this site. This, of course, implies a passive blood stream invasion, with the biliary tract as a seeding focus in this instance.

The known blood stream invasion in



FIG. 5.—Case 2. Ileocecal region showing hyperplastic hemorrhagic mesenteric nodes and congested mucosa. (Approx. $\times \frac{1}{2}$.)

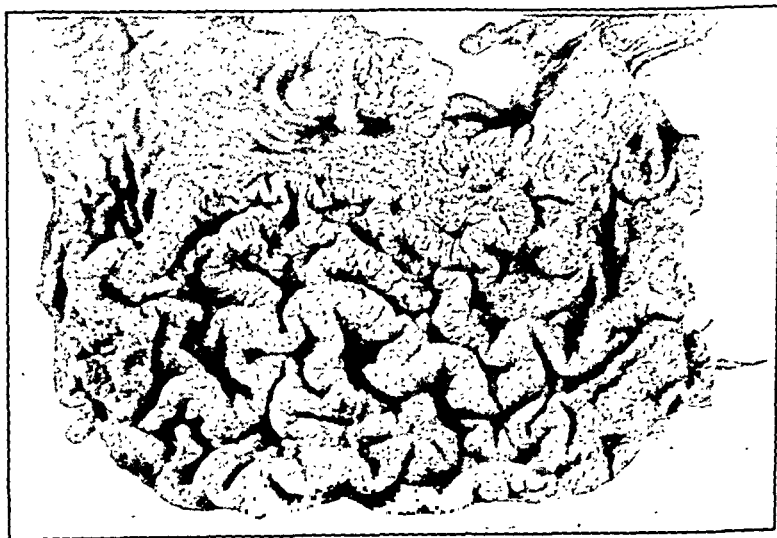


FIG. 6.—Case 7. Stomach showing marked acute diffuse gastritis. (Approx. $\times \frac{1}{2}$.)

experimental *Salmonella* infection studies, and its independence of the portal of entry, parallels these observations. In the experimental animals, bronchitis and bronchopneumonia and cholangitis are very common. The pathogenesis of the enteric lesions as indicated by the experimental work of Orskov and co-workers,^{8,9,10} Seiffert and co-workers,¹⁶ Takita,¹⁸ Waldman,^{19,20} and Müller⁶ renders the variable localization and mutable clinical picture explicable. Much of the experimental work was carried out with *S. typhi murium* in mice. It was established that the microorganism very rapidly invaded

animals, to be presented in detail elsewhere, nearly all lesions seen in the human autopsy series were encountered. The typhoid lesions in the gut could be produced with regularity by controlling dosage and method of administration. The cholangitis, and the lymph node and spleen involvement were common. The kidney localization did not seem to occur, though others have noted this finding.

The only instance in the literature in which the typhoid-like morphology of the *Salmonella* lesions in a human case was adequately described was reported by Neesen and Merkel,⁷ who found 1 such



FIG. 7.—Case 8. Showing fibrinopurulent pericarditis. (Approx. $\times 1$.)

the blood stream, to be excreted in the bile. The lesions in the gastro-intestinal tract, with the localization in lymphoid nodules and Peyer's patches, really represent secondary lesions, rather than a primary portal of entry as first considered. It is this period of early or immediate generalization, with its immunologic significance, that gives the opportunities for the variations encountered.

In a report on experimental studies in

case in a group of 4 deaths due to paratyphoid Breslau (old terminology for *S. typhi murium*).

It was felt that this tendency to localization in the Peyer's patches in the intestine might be accountable on the basis of strain variation. To test this hypothesis, several strains of *S. typhi murium* were fed to laboratory animals: at first guinea pigs, rats and mice, and then guinea pigs and mice only. In all, 6 strains of *S. typhi*

murium were used for this purpose (Grier, Thomas, 1182 B.I., Kaufman, B.I. 43930 N.Y.S. lab., Williams). The initial group of guinea pigs fed the strain of micro-organisms recovered from this initial case (Grier) showed remarkable localization of involvement in the region of Peyer's patches and mesenteric lymph nodes, in contrast to some of the other strains used for comparison (43930, Williams). The findings in the remaining viscera are not being considered here but showed the corresponding visceral typhoid-like lesions. These original observations have not been corroborated and have been corrected by subsequent experiments in guinea pigs, which showed that several other *Salmonella* strains (*S. typhi murium*, Kaufman B.I., enteritidis and Thomas) can produce such lesions. The detailed analysis of our failure to correlate serologic antigenic structure with the form of reaction will be given separately. It may be stated that the method of administration is most important, and that placing favorable doses of organisms in the food favors localization of the lesions in Peyer's patches.

Biology. Several members in the *Salmonella* group show a remarkable selective specificity for particular hosts while others, though they have preferential hosts, may also infect other species, but usually producing a milder infection. A microorganism like *S. typhi murium* has a wide host range with a wide variation in reaction and symptomatology extending from the carrier state to fulminating infection with death. This bacterium has become most widespread in nature with the widest range of pathogenic adaptability to multiple hosts. The phenomena of variation and adaptation for bacteria are best studied in this group (Savage¹⁵). The general intermediary position of *S. typhi murium* infections between the septic picture of *S. cholerae suis* and the enteric picture of *S. oranienberg* was obvious in our own clinical material by the numerical incidence of the characteristic clinical features, and from the standpoint of general clinical

impression, as well as by the morbid changes and the laboratory data. Our experimental studies confirm the ready adaptation of this organism for the study of the multifarious forms of reaction of the enteric group.

The members of *Salmonella* group show interesting serologic relationships to known pathogens such as *E. typhosa* as well as to variants of the originally non-pathogenic *B. coli* group (Saphra and Silberberg¹⁴). A complete analysis of our own unavailing attempts to conciliate morphologic changes produced by the *Salmonella* on the basis of serologic analysis will be recorded elsewhere. This is in keeping with the experience of others: Boivin,¹² Pike and MacKenzie,¹¹ and Pike and Swinney,¹² and with the expressed opinion of Seligmann and Saphra.

The biochemical features showing gradation and transition between the *Salmonella* group are better known. We have encountered several gasless *S. typhi murium* strains. The original identification in each instance was typhoid by us and by the New York City Department of Health, but complete antigenic study by Dr. Seligmann and Dr. Saphra established the correct identification as a variant of *S. typhi murium* which failed to produce gas under the normal controlled conditions.

We wish to record 2 cases with severe gastro-intestinal symptoms, clinically suspected of being caused by *Salmonella* infection, in which atypical forms of colon bacilli were recovered. In the first instance, a pathogenic rôle is suggestive; in the second case, the ulcerative lesions are often found above an obstruction. This probably represents a common finding and the atypical coli form of bacillus may be a mere coincidence.

Clinical Notes. CASE A-45-34: The patient was admitted with an acute abdominal episode and considered to be mesenteric thrombosis or preëxisting duodenal ulcer. Severe epigastric pain was the outstanding finding. At autopsy, severe gastritis and enteritis were found. Only an atypical coli

organism was recovered, present in great numbers.

CASE A-40-58: A 65 year old female admitted with intestinal obstruction died with an episode of acute dyspnea and abdominal pain. The autopsy showed extensive deep ulcerative lesions of the colon above an obstructing carcinoma. Bacteriologic study revealed an atypical coli organism and a streptococcus non-hemolyticus.

We do not mean to imply that an atypical colon bacillus is invariably a pathogenic microorganism. Others have suggested that this bacterium can produce symptoms and lesions. The existence of transitional biochemical and serologic phenomena between atypical colon bacilli and the Salmonella group might lead us to expect pathogenic strains of atypical *B. coli* to occur on theoretical grounds.

We attempted studies of the pathogenicity of the atypical Coli organisms recovered from these 2 cases in guinea pigs and mice by feeding experiments, but obtained no evidence of infection in the form of any lesions or deaths. We do suspect that transition forms of *B. coli* will be found which will be pathogenic for some humans and animals on ingestion.

Epidemiology. Though all of the material represents sporadic cases, several interesting epidemiologic observations were encountered in this survey. Several double infections were encountered. Two nurses with *B. oranienberg* showed, in addition, a superimposed *S. manhattan* and *S. typhi murium* respectively. One baby showed a double infection with *S. morbificans boris* and *S. muenchen*.

Some of the types encountered, as *S. morbificans boris*, *S. panama* and *S. lichtfeld*, are distinctly rare. Our attempts at epidemiologic analysis were disappointing. At one time we had 6 premature infants showing *S. morbificans boris* in their stools. Three of these infants showed no symptoms whatsoever. The other 3 showed mild diarrhea. None was severely ill. No source for this epidemic could be established in the hospital staff on the maternity ward or in the dietary division.

One patient was admitted with vomiting, high fever and diarrhea, and *S. typhi murium* was obtained in the stool. The patient was discharged as cured after 4 weeks of illness. The sister of this child was admitted 4 days after the onset of her sister's symptoms for an incidental surgical repair of an umbilical hernia. A stool culture showed this same organism, *S. typhi murium* but no symptoms were present and no history relative to such a previous infection could be obtained.

One student nurse developed a diarrhea, and *S. oranienberg* was recovered. The patient was discharged within 3 days. A survey showed that a waitress in the nurses' dining room had this organism in her stool. This waitress admitted a bout of diarrhea a short time previously.

Summary and Conclusions. During a 4 year period at the Queens General Hospital, 86 sporadic instances of Salmonellosis, revealing 21 known serologically identifiable strains were encountered. The study includes a tabular correlation of the nature and severity of the clinical picture, and the bacteriologic data, with particular reference to the sources of the cultures.

The 3 most common species, *S. cholerae suis*, *S. typhi murium* and *S. oranienberg*, are compared. The carrier state, the enteric fever form with and without gastro-intestinal manifestations, and the septic forms alone and in combination, are presented from the standpoint of their individual features and their inter-relationships. The syndromes presented by the 13 cases who died, and the pathologic findings in the 9 instances examined at autopsy, are tabulated, with a correlation attempted with the form of the clinical illness, the bacteriologic data and the morbid changes encountered.

The intermediary position of *S. typhi murium* in this comparison, from the standpoint of the clinical picture, the severity of the illness and the morphology of the lesions, is stressed, and the biologic implications noted. The data from 17 *S. typhi murium* deaths on file at the Beth Israel Hospital Salmonella Typing

Center and the autopsy findings in 9 of them are tabulated for comparison.

The problem of the adaptation of the *Salmonella* organisms to different hosts, and the variable course the infection pursues in the different and in the same hosts, is discussed. The variation in the clinical course is stressed. Reference to the lesions of experimental *Salmonella* infection under varying conditions, appearing in a separate communication, is included for comparison with the human morbid pathology. An analysis of the clinical

course and the pathologic data is presented in the light of experimental studies of the pathogenesis of the enteric disease group, and the resemblance to typhoid fever in its mutable and multifarious forms. Typhoid-like lesions at autopsy caused by *S. typhi murium* are emphasized. The broad biologic problem of Salmonellosis is presented from the standpoint of its relationship to the atypical coli infections of the gastro-intestinal tract on the one hand, with 2 instances cited, and with typhoid infections on the other.

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HOOKWORM INFECTIONS IN TROOPS RETURNING FROM THE PACIFIC

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ROUTINE stool surveys of men returning to this country from various Pacific Islands have disclosed, among other parasites, a large number of hookworm infections. Of 2500 consecutive admissions to this hospital from the Pacific Theatre, 11.5% were found to have hookworm eggs in the stool. By contrast, the incidence of hookworm infestation in 4300 patients who had seen only continental service in this country was 6.2%.* The composition of both groups from the standpoint of residence in southern hookworm states is sufficiently uniform so that this factor is not responsible for the difference observed. The complete details of those surveys are being reported in another paper (Lt. Col. T. B. Wilson, M.C., to be published).

The purpose of this paper is to present observations on the nature, severity and results of treatment in patients with hookworm infection who had served in the Pacific Theatre and who had been evacuated for other reasons.

Material and Methods. This report deals with 284 patients. In 210 the diagnosis of hookworm infection had been made overseas while in 74, eggs were first demonstrated at this hospital. The complete clinical records of the patients in whom hookworm eggs had been found in the stools overseas or in other hospitals in this country were reviewed to determine the total number of treatments they had received prior to admission to our Tropical Disease Section. If hookworm eggs were found on admission, all previous treatments were considered failures, but if the stools were consistently

negative the last treatment for a positive stool was considered successful. All patients in this series with positive stools at this hospital received one or more courses of treatment, not on clinical grounds, but to determine if possible the genus of the hookworm which they harbored as well as the efficacy of treatment.

Each stool was examined at least once by direct smear, zinc sulfate centrifugal flotation, brine flotation, and water centrifugal sedimentation. Stoll egg counts were done before treatment in 75 patients. The routine treatment employed was as follows: 30 gm. of sodium sulfate were given after the evening meal the night before treatment. No breakfast was allowed, and 3 cc. (occasionally 5 cc.) of tetrachlorethylene given in soft capsules. Two hours later another 30 gm. of sodium sulfate were given. Following treatment, all stools were collected directly into a large wide-mouthed jar until 4 p.m. of the day of treatment. The stool was then washed through a metal sieve and if adults were recovered, they were identified by the morphology of the mouth parts. The total number, genus and sexes were recorded.

Results. 1. *Severity of Infection.* The average Stoll count on admission in 17 patients who had been treated on 1 or more occasions overseas and subsequently were found here to have *Necator* infections was 1900 eggs per gm. of feces, with a range of from 100 to 9100. The average Stoll count on admission in 28 patients who had been treated on 1 or more occasions overseas and subsequently were found here to have *Ancylostoma* infections was 2295 eggs per gm., with a range

* This figure is, however, not to be regarded as representative of the nation-wide picture, for there is evidence to suggest that men from northern sections acquired their infection while in southern training stations.—THE EDITORS.

of from 200 to 9800. These findings may not reflect the original degree of infection, since all these patients had had 1 or more courses of treatment prior to admission here. However, the average Stoll counts prior to treatment in 15 patients in whom eggs were first discovered at this hospital and who subsequently were shown to harbor *Necator* or *Ancylostoma* were 2400 and 3200, respectively. This, as well as the total number of worms recovered subsequently (see below) indicates that the hookworm infections acquired overseas in the majority of patients were very light.

2. *Adult Recoveries.* Following treatment, adult hookworms were recovered from 169 patients. The ratio of female to male for *Necator* was 1.6 and for *Ancylostoma* 2.3. The total number of worms as well as the genus recovered from these patients is summarized in Table 1.

TABLE 1.—RECOVERY OF ADULT HOOKWORMS FOLLOWING TREATMENT WITH TETRACHLORETHYLENE IN 169 PATIENTS

	No. patients	No. of adults recovered					
		1-5	6-10	11-25	26-50	51-100	101-150
<i>Necator</i>	69	41	12	10	6		
<i>Ancylostoma</i>	87	56	15	10	4	2	
Mixed	13	4	3	4	2
Total	169	101	30	24	10	2	2

The maximum number of *Necator* was 86 and of *Ancylostoma* 112. In only 14 of the 169 patients from whom adults were recovered were more than 25 worms found. Following treatment of 36 patients no adults were recovered although repeated stool examinations subsequently were negative for eggs. Failure to find adults in these patients who were apparently cured of the infection is regarded as evidence that the number of worms they harbored was very small, although it is admitted that the duration of collection may not have been long enough to find them. This also applies to 79 patients from whom no adults were recovered after treatment, although subsequent stools were positive, indicating treatment failure. Thus, on the basis of the total number of worms recovered following

treatment as well as the Stoll egg counts, it seems evident that the hookworm infections in the majority of these men are relatively light.

It is important to note that 65% of the patients from whom adults were recovered harbored *Ancylostoma*. Only 12% of the latter resided in southern states or had received military training in possible hookworm areas in this country. On the other hand, 75% of the men with *Necator* infections resided in southern states in this country. It seems probable, therefore, that the increased incidence of hookworm among men returning from the Pacific is due largely to the acquisition of *Ancylostoma* infections overseas and that the ratio of *Ancylostoma* to *Necator* acquired in the Pacific is at least 3:1.

3. *Efficacy of Treatment.* Tetrachlorethylene has been reported as producing cures in 70 to 90% of patients with hook-

worm infections following a single course of treatment with this drug. In our experience the results of treatment judged by the number of courses administered to the same patients overseas who still had positive stools on admission here are not nearly as encouraging. Of 100 patients who received one or more courses of tetrachlorethylene overseas, only 42% were cured of their infection after the first course of treatment as determined by repeated stool examinations at this hospital. The results of multiple treatment overseas in these patients whose records were complete are presented in Table 2.

Since no adult recoveries were done following these treatments, no conclusions can be drawn concerning the relative efficiency of tetrachlorethylene in eliminating *Necator* versus *Ancylostoma* infec-

tions. However, since 65% of adults recovered from 169 patients were *Ancylostoma*, the relatively poor results of multiple treatment overseas may be due to the persistence of this parasite. In this connection, it is of interest that of 35 patients in whom eggs were first discovered at this hospital, and who were treated for the first time for hookworm infections which were subsequently proved to be *Ancylostoma*, only 9 (25%) were cured after the first course of drug; 18 patients were re-treated and 7 (39%) were cured, making a total of 55% cured after 2 courses of treatment. On the other hand, of 24 patients diagnosed and treated for the first time at this hospital for infections subsequently proved to be *Necator*, 16 (66%) were cured. Three out of 5 who were re-treated were cured, or a total of 85% cured after 2 courses of treatment. These observations, while limited in number, suggest that multiple treatments overseas because of repeatedly positive stools were the result of *Ancylostoma* infections in the majority of such patients and that tetrachlorethylene is less effective against *Ancylostoma* than *Necator*. The relatively low incidence of double infections with *Necator* and *Ancylostoma* in returnees from the Pacific (13 cases in 169 patients) may be due not only to a lower incidence of *Necator* in some Pacific areas but to elimination of *Necator* from

a large proportion of the men as a result of several courses of treatment prior to their return to this country.

4. *Other Parasites.* Hookworm stools frequently had additional parasites. Of 197 patients who had hookworm eggs in the stools, 80 had 1 or more helminths, 59 had 1 or more protozoa, and 22 had both. The incidence of *E. histolytica* in this group was 11%. The occurrence of *S. japonicum* is a reflection of the fact that many of the patients in the group had served on Leyte and had been returned because of proven or suspected schistosomiasis; the figure shown, therefore, cannot be taken as indicating the incidence of this infection in the Pacific. The distribution of parasites other than hookworm in this group is shown in Table 3.

5. *Clinical Aspects.* The clinical records of 100 malaria and surgical patients with hookworm as the only helminthic infection were reviewed. In no case were any signs or symptoms found which could be attributed to hookworm infection alone. The average red cell count was 4.2 million per c.mm., the lowest being 3.3 million. Only 10 patients had less than 4 million red cells and in these the anemia appeared to be related to a recent attack of vivax malaria or a chronic surgical infection rather than hookworm infection. The average percentage of eosinophils in this group was 10.2. In our experience hook-

TABLE 2.—EFFICIENCY OF REPEATED COURSES OF TETRACHLORETHYLENE ADMINISTERED OVERSEAS IN 100 RANDOM PATIENTS WITH HOOKWORM INFECTIONS

Based on Complete Clinical Records and Stool Examinations on Admission—Includes *Ancylostoma*, *Necator* and Mixed Infections

No. treatments	No. treated	Subsequent stools		
		Positive	Negative	Total % cured
1	100	58	42	42 0
2	48	34	14	62 2
3	30	18	12	79 1
4	16	12	4	85 7
5	8	4	4	95 0
6	3	1	2	95 7

TABLE 3.—DISTRIBUTION OF OTHER PARASITES IN 197 PATIENTS WITH HOOKWORM INFECTIONS

	No.	%		No.	%
<i>E. histolytica</i>	22	11	<i>S. japonicum</i>	68	34
<i>E. nana</i>	41	20	<i>T. trichiurus</i>	26	13
<i>E. coli</i>	21	10	<i>S. stercorale</i>	7	4
<i>I. fasskii</i>	11	5	<i>A. lumbricoides</i>	4	2
<i>G. lamblia</i>	8	4			

worm infection in service men returning from overseas are not clinical and do not represent hookworm disease.

Summary and Conclusions. 1. The incidence of hookworm infection in troops returning from the Pacific is 1.8 times that found in troops who have served only in this country.

2. Of 169 infections in which the type of hookworm was determined, 65% were due to *Ancylostoma*. The increased incidence of hookworm infections found in service men returning from various Pacific islands is believed largely due to the acquisition of *Ancylostoma* overseas.

3. In our experience tetrachlorethylene is not as efficient in eliminating *Ancylostoma* as it is in *Necator* infections, and this may account for persistently positive

stools after multiple courses of treatment overseas.

4. The demonstration of a high proportion of *Ancylostoma* infections in men returning from the Pacific may be of concern to public health authorities in this country, in view of the possibility of the introduction and establishment of this parasite.

5. Hookworm infections in the majority of returning servicemen are light and rarely represent hookworm disease.

6. Multiple parasitism is common in men returning from the Pacific. Finding 1 or more parasites, though these may be non-pathogenic, should stimulate continued search so that potentially serious infections are not overlooked.

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PROGRESS OF MEDICAL SCIENCE

PATHOLOGY AND BACTERIOLOGY

UNDER THE CHARGE OF

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FIBROCYSTIC DISEASE OF THE PANCREAS

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It is unfortunate that the name, fibrocystic disease of the pancreas, is a purely descriptive pathological term, the use of which is necessitated by our lack of knowledge regarding the etiology of the disease. In the period prior to 1938, individual cases were reported under many and varying descriptive terms. The synonyms are as follows: pancreatitis, pancreatic disease, atrophy of the pancreas, congenital pancreatic steatorrhea, celiac disease, congenital cystic fibromatosis of the pancreas, agenesis of the exocrine portion of the pancreas, congenital pancreatic disease, cirrhosis of the pancreas, congenital familial steatorrhea, chronic interstitial pancreatitis in infancy and pancreatic insufficiency (Wolman).⁷³² As is readily seen, many of these terms are of clinical origin and arose during the twenty odd years before the appearance of Dorothy Andersen's classical article in 1938,¹² clarifying and correlating the clinico-pathological relationships. As will be understood, when the pathological findings and etiological factors are discussed, the name fibrocystic disease is not satisfactory but a more adequate one is lack-

ing. Although the cysts are mainly microscopic, it seems unwise to call it simply fibrosis of the pancreas as the latter appears to be secondary to the acinar lesions. Besides, fibrosis is a marked feature of congenital syphilis (Porter⁵¹), and this term might be confusing.

The clinical, biochemical and pathologic findings are closely inter-related in this disease and to reach an understanding of the pathogenesis and etiology, it will be necessary to touch briefly on the clinical and biochemical aspects. But within the space of this review, it will be impossible to enter into all of the clinical details which may be found in other publications referred to below in their appropriate places.

It must be noted before proceeding further that the pancreatic changes give rise to two clinical entities:¹² meconium ileus of the newborn and the syndrome of pancreatic steatorrhea in later life. In the former, the pancreatic changes are well advanced at birth, while in the latter, the changes are slowly progressive over months or years.

HISTORICAL. It is of interest to sketch

the historical background of the disease as it illustrates the long period that may elapse, in a relatively common disease with typical pathological findings, before the point of supersaturation is reached and a single observer gathers the evidence together and crystallizes the clinical and pathological findings into a disease entity. Prior to Andersen's paper,^{1a} published in 1938, about 27 cases had been reported under a variety of names. In the 8 years elapsing since 1938 several hundred have been reported. Furthermore, Andersen's article has stimulated a renewed interest in the exocrine pancreatic secretions which had been previously over-shadowed by its endocrine function. This was in part due to the difficulty of estimating pancreatic enzymes, of interpreting any changes noted and lastly to the fact that adult pancreases do not commonly show lesions of the exocrine portion of the gland, except in association with obstruction of the pancreatic ducts by stones or carcinoma or in acute pancreatic necrosis.^{4,11a,47,60,61}

Much of the delay in establishing this disease as an entity was caused by writers clinically regarding these cases as Gee-Herter's (celiac) disease and the apparent failure of observers to note the isolated case reports appearing in the literature of proven advanced pancreatic disease with clinical symptoms of celiac disease. Parsons,⁵⁰ in his Rachford lectures in 1932, stated that the pancreas is normal in celiac disease, which is, of course, true, but he makes no mention of the existence of pancreatic deficiency disease. Undoubtedly some clinicians were on the right track but failed to report adequately such pathological material as they obtained or interpreted the findings purely as secondary lesions.^{55,67} Some cases were buried in the literature on vitamin A.^{7,72}

Undoubtedly, the pathologist was at fault in not taking routine autopsy sections of that unassuming organ, the pancreas. In a marasmic infant or a child with bronchopneumonia, there would appear to be sufficient cause of death, without taking sections of all organs. Further-

more, because many of these infants die under 1 year of age, the clinician failed to note the intestinal symptoms, so frequently overshadowed by the respiratory disease. The paucity of cases reported prior to 1938 would seem to be accounted for in this manner. Awareness of the disease has markedly increased its reported incidence.

The pancreatic lesions were probably first described by Landsteiner⁴² in 1905 but his was a case of meconium ileus and probably, therefore, was ignored. Between 1905 and 1919 several suggestive clinical cases were published but without post-mortem examination.⁸ These were followed by isolated reports with pathologic studies in the literature up until 1937.^{5,7,12,13,15,23,28,30,32a,33,35,41,49,64,69,72}

Although Parmelee⁴⁹ separated his cases from true celiac disease and apparently had a clear understanding of his cases, they were too few in number to make an impression on the pediatric world. The same probably applies to Harper's report in 1930^{32a} and 1938.^{32b} Finally, in 1938, Andersen^{1a} clarified the status of the disease in a report on the findings in 22 new cases plus the above 27 cases and gave a clear description of the clinical and pathological aspects. In the same year Harper,^{32b} Thomas and Schlutz,⁶⁸ and Blackfan and May,⁶ in a series of independent observations, also confirmed Andersen's conclusions.

Since 1938 numerous cases^{2,16,18,24c,26,27,-34,45,52,58,59} have been reported but relatively little or no advance has been made on the problem of etiology. Improved techniques for obtaining and estimating the pancreatic enzymes, resulting in more accurate clinical diagnoses, have constituted the main advance. Treatment of the disease, studies of stool and blood lipids, detailed pathologic anatomy and one attempt to reproduce the disease, have been the principal subjects of investigation.

CLINICAL ASPECTS. The disease clinically can be almost identical, in symptomatology, with idiopathic celiac disease so far as the gastro-intestinal system is concerned, the main differences being in

the earlier age of onset and the high incidence of suppurative lung lesions and the fatal outcome in fibrocystic disease of the pancreas.

In most cases the time of onset is at birth or within the first 6 months to 1 year of life.^{1a,52} Although the severity of the pathologic changes in the pancreas vary considerably in different cases, no attempt to correlate the age of onset or severity of symptoms with the pancreatic changes has been made. The age at death varies somewhat in the series reported but ranges from 2 days to 14½ years.^{1a,5,52} About 70 % of patients die during the first year of life, mainly as the result of respiratory disease. A small percentage survive beyond this period but the eventual outcome is fatal.

The symptoms are marked by their variability but the cases may be divided into: (1) those with predominant respiratory symptoms; (2) those with predominant gastro-intestinal symptoms, and (3) those with a combination of both respiratory and intestinal disturbances.⁵²

Andersen^{1a} divided her cases in 3 groups and this seems to have been followed by subsequent observers. These are: Group 1—patients dying in the neonatal period, usually with meconium ileus with absence of other gastro-intestinal symptoms or lung infection. Group 2—patients dying within the first 6 months of life (later observers have extended this group to 1 year) and having nutritional disturbances often obscured by respiratory symptoms. Group 3—patients dying after 1 year of age with gastro-intestinal symptoms resembling celiac disease.

Andersen's second and third groups are characterized by respiratory symptoms, emaciation and under-development in spite of an excellent appetite. The stools may appear to be normal in early infancy, in many cases, but with the giving of solid foods the stools become large.^{1a,1c} The variation in the description of the stools by different writers is probably due to the effects of multiple factors influencing digestion and absorption, which vary from patient to patient.

All cases give a history of cough, sooner or later; the onset varying from 2 weeks⁷ to upwards of 11 years.⁴⁹ It progressively becomes more severe and eventually patients develop purulent bronchitis, bronchiectasis and pneumonia.

INCIDENCE. The incidence of this disease, as found at autopsy in children, varies somewhat from author to author. Andersen^{1a} reported an incidence of 3.3 % in 605 autopsies, Menten and Middleton⁴⁵ reported an incidence of 2.8 % between 1936 and 1943 but could find no cases in their autopsy material for the 10 years prior to 1936. Blackfan and May⁶ reported 1.3 % and Farber^{21b} 4.8 % between 1937 and 1942. The latter author found an incidence of 12 % in 1942. Some authors^{45,65} consider that the disease is more prevalent than formerly. Such opinions do not seem justifiable at the present time as more and more cases with less marked lesions are being diagnosed. For example, Andersen^{1a} reported only those cases with 90 % or more of functionless pancreatic tissue yet states that 11.4 % of the pancreases examined by her showed less marked lesions. One may conclude that the true incidence is not yet established but may be in the region of 4 % or more of autopsies.

Andersen^{1a} gave the sex incidence in her series as about 67 % females and 33 % males, suggesting that females were more frequently affected. Since then, it has become apparent that there is no sex predominance in fibrocystic disease of the pancreas.⁵²

The familial incidence is relatively high as reported by Howard³¹ in 1944. He reviewed 120 cases and found 12 families in which each of 2 children had had the disease. Several authors have reported the disease as occurring in twins, sometimes affecting only one twin,^{12,27,35} at other times both twins.^{6,7} None of the twins appeared to be identical. No studies have been done with reference to the Mendelian principles.

RACE AND DISTRIBUTION. Cases have been reported from Australia,²³ Canada,^{25,52} England,¹² Germany,⁵ and the Hol-

land,³⁵ New Zealand,¹⁸ and the United States. The parents may come of Italian, German, Irish, English, Scotch and American stocks.^{1a,45} Whites would seem to predominate as Andersen reports only 1^a and Menten and Middleton⁴⁵ only 2 cases occurring in Negroes.

ROENTGENOLOGICAL ASPECTS. The features of this diagnostic aspect have recently been reviewed by Pugh⁵⁷ in this journal. Roentgenologic studies indicate that there are non-specific intestinal changes and that the bilateral, widespread lung changes are suggestive but not pathognomonic of fibrocystic disease.

PATHOLOGIC ANATOMY. Externally the body is characteristically underdeveloped and emaciated with gross evidence of muscular atrophy. The abdomen is often protuberant and there is frequently an umbilical hernia. The body cavities are usually normal except that the pleural cavities may show evidence of inflammation, as would be expected from the incidence of lung lesions.

PANCREAS. The pathognomonic lesions essential to pathologic diagnosis are found in the pancreas and it is on the basis of the pancreatic changes that the disease entity has been distinguished from others. It must be emphasized that so far as can be judged from the histopathology of the pancreas, the lesion is essentially one of acini and ductules. The fibrosis is a secondary phenomenon upon which too much emphasis has been laid and which unfortunately is included in the cognomen of the disease for lack of a better name. This fact has been pointed out by Farber^{24c} but needs to be re-emphasized. The secondary character of the fibrosis is clearly evident from the appearance of the milder lesions in which there may be little or no fibrosis but definite acinar-duct changes. Thus the lesion is essentially one of the exocrine portion of the gland.

Grossly, the organ may or may not show changes,^{1a,24c} depending on the extent of the lesion and possibly also on the duration. Farber^{24c} has given the most extensive pathologic report on the pancreas

with 87 cases as material. As Andersen^{1a} has stated, accurate gross diagnosis is markedly improved by experience. The pancreas is usually firmer, smaller and thinner than normal. The weight is less than normal, although it must be remembered that there is a wide variation in normal weights. Mitchell and Nelson⁴⁶ give the following normal values: New-born and up to about 2 months of age, 2 to 3.5 gm., 1 year of age, about 10 gm., 4 to 5 years, 20 gm., and 10 to 12 years, 30 gm.

In those cases with advanced fibrosis there may be a distinct grittiness on cutting into the pancreas. If there is marked cyst formation and advanced fibrosis, the organ will present an irregular furrowed appearance due to alternating depressed fibrotic areas and elevated cystic or uninvolved normal pancreatic tissue. The cut surface will exhibit irregular lobulation with intersecting wide bands of connective tissue, as opposed to the regular, normal diamond-shaped pattern with scarcely visible septa. In some cases the cut surface, particularly in the head of the gland, will show dilated ducts filled with yellow plugs. Cysts visible to the naked eye are not often present. Occasionally the pancreas is grossly fatty.

The patency of the main pancreatic ducts is difficult to ascertain with any degree of accuracy but the consensus is that they are patent in the majority of patients living beyond the neonatal period. The incidence of atresia in meconium ileus is higher. It is extremely difficult to dissect the main ducts in infancy due to their small size. Farber^{24c} states that in his series, a few cases showed definite narrowing of the ducts but in the majority no congenital atresia or stenosis was found. There was stenosis in Kornblith and Otani's case⁴¹ and in Hurwitt and Arnheim's³⁶ as proven by following the duct of Wirsung in serial sections. Cases of stenosis are reported by Benoit⁵ and Tiling.⁶⁹ In Andersen's^{1a} own cases attempts to demonstrate the patency of the ducts were unsuccessful in 4 out of 5 cases;

the lumen appeared to end in fibrous tissue 5 to 15 mm. from the ampulla of Vater. In the other case the pancreatic duct was dilated throughout.

An important argument in favor of patency of the ducts is put forward by Farber.^{24c} A history of meconium ileus is not given by those patients who survive the newborn period. If congenital obstruction of the ducts were the cause of pancreatic fibrosis, then meconium ileus should be a constant finding. This has not proved to be the case. However, Oppenheimer's case¹⁸ would seem to contradict this statement, as duct atresia was found in a 10 months' old infant who had no history of meconium ileus.

Microscopically, the essential lesion is accumulation of an eosinophilic secretion in the acini and small and large ducts. The eosinophilic material is homogeneous and frequently lamellated, more particularly in the larger accumulations. Some of the larger masses contain fragments of eosinophilic substance mixed with pale faintly yellow material. This material does not give the staining reaction of mucus by mucicarmin methods⁷¹ but on the other hand, Baggenstoss and Kennedy² state that it stains like mucus and occasionally like fibrin when appropriate stains are used. Occasionally the material filling the ducts is calcified. Sometimes desquamated epithelial cells, occasional macrophages or rare polymorphonuclear leukocytes are seen in the lumens. In the case reported by Gamble²⁷ the lumens were reported to contain many leukocytes.

The acini and ducts are dilated to a size that varies with the amount of secretion present and the duration of the disease. In the younger infants, large cysts are not common. Although one speaks of cystic disease of the pancreas, the cysts are essentially microscopic. The lining epithelium may vary from normal height to very low, the degree of flattening apparently depending on the amount of accumulated secretion. Hess and Saphir²² report the finding of occasional mitotic figures in the acini. They also state that

the cells were granular and the nucleus indistinct.

The accumulation of secretion in the ducts has been reported in only a few other diseases. Rich and Duff,⁶⁰ reporting on acute pancreatic necrosis, state that in 3 of their cases the disease may have been due to the presence of an abnormal secretion, since numerous ducts contained inspissated secretion that stained deeply and irregularly with eosin. In these cases the masses filled the ducts or appeared as individual masses separated by thinner secretion. Where the epithelium was desquamated, the duct wall was destroyed and infiltrated with an abundant polymorphonuclear exudate. This latter phenomenon has not been observed in fibrocystic disease and suggests that the secretion lacks trypsin, as active sterile pancreatic juice calls forth a polymorphonuclear exudate.⁶⁰

Apparently the accumulation of secretion in fibrocystic disease results in pressure atrophy and gradual disappearance of the acinar tissue. In extreme cases all that remains are isolated, widely separated, abnormal lobules and ducts scattered throughout the pancreas, with almost complete disappearance of acini.

The islets of Langerhans are not affected as a rule, although they were thought to be decreased in several cases^{30,35,51,62} and abnormal in one report.² Andersen^{1a} states that in some cases they were seen to maintain their connection with adjacent acini or ducts but showed no other change. These histological findings are compatible with the absence of hyperglycemia and glycosuria in this disease but the finding in some cases of a high glucose tolerance curve suggests that the histopathology of the islets requires more detailed investigation.

The supporting tissue of the pancreas is, in the later stages, greatly increased and the organ appears fibrotic. It is both interlobular and intralobular (peri-acinar). There is no definite evidence of active production of collagen and fibroblasts are uncommon. According to Farber^{24c} the

apparent increase is due to coalescence of the fibrous stroma following atrophy and disappearance of acinar tissue. He also states that in his series neither necrosis nor acute inflammation were seen, although in some cases, the stroma was infiltrated by large mononuclear cells and lymphocytes. Andersen^{1a} makes the statement that these inflammatory cells varied from a few to a sufficient number to warrant the diagnosis of subacute or chronic pancreatitis. When the cells were numerous a few polymorphonuclear leukocytes were present as well. The cellular infiltrations were diffuse and bore no consistent relationship to the ducts. The mononuclear exudate is probably associated with products of degeneration, rather than with the presence of infection. Such infiltrations have been noted to be marked shortly after ligation of the pancreatic ducts in animals but they decrease as the pancreas becomes more and more atrophic and fibrotic.^{4,29}

Search of the literature has failed to reveal any adequate explanation or description of the exact mode of disappearance of the acinar cells or the masses of inspissated secretion. The latter are presumably absorbed as no foreign body reaction has been noted, nor have the concretions been seen elsewhere than in the lumens of the ducts or acini.

Those cases showing minor lesions are perhaps of most interest and of greatest significance. As Farber^{24c} states, they show no, or slight, increase of connective tissue and the large ducts are not dilated but the lumens of the small ducts and the acini contain acidophilic inspissated secretion and are dilated. In some of these earlier cases, the inspissated secretion may be present in some lobules and absent in others.⁷¹ Furthermore, considerable numbers of normal acinar cells with acidophilic secretory granules may be seen.^{1a,71}

A few cases have been reported^{13,17,28,30,32b,64,68,71} in which the pancreas is largely fatty, only remnants of ducts remaining surrounded by localized areas of fibrous tissue. The fat is of the ordinary type.

This occurs mainly in the older age groups. It is presumed from the clinical history and autopsy findings that these changes are similar in origin to fibrocystic disease and are simply a far advanced stage of the disease. This finding is by no means incompatible with prolonged obstruction of pancreas, as in pancreatic lithiasis and carcinoma of the head of the pancreas⁴⁷ such may occur. Also, in experimental ligation of ducts in animals,⁴ fatty infiltration has been reported. On the other hand it is also found in obese subjects without obstruction.⁷⁰ It is difficult to understand why, in a disease marked by disappearance of fat from the depots of the body, the pancreas in a few cases is extensively replaced by fat.

RESPIRATORY SYSTEM. Lesions of the lungs are almost invariably present in those patients who live beyond a few weeks. Grossly, there may be hyperexpansion of the lungs, particularly anteriorly, associated with obstructive atelectasis elsewhere. The trachea, bronchi and bronchioles are obstructed by a thick tenacious mucoid or muco-purulent exudate.^{24c} The latter in some cases is abundant. There is moderate to marked bronchiectasia and bronchiolectasia, depending on the duration of the disease.

Bronchopneumonia and abscess formation are common. In most of the cases^{1a} the abscesses appear to arise in relation to the small bronchi. The bronchial and bronchiolar walls are infiltrated with inflammatory cells. Blackfan and May⁶ refer to these infiltrating cells as being of the mononuclear and lymphocytic type, while the lumens are filled with polymorphonuclear leukocytes. In those cases with vitamin A deficiency, keratinizing squamous metaplasia of varying degree may be present in the bronchi and trachea. Where the process is of long duration, considerable fibrosis of the lungs may occur and there may also be a chronic interstitial pneumonia.^{21c} Blackfan and May⁶ state that the pneumonia is primarily an interstitial one with secondary involvement of the alveoli and alveolar ducts.

The common lung invader is the staphylococcus pyogenes. Farber^{24c} states that this organism appears to be of low virulence, as shown by its lack of invasiveness and by the relatively small amount of damage to the lungs, even after a process lasting for months. Farber^{24c} is the only author who appears to have drawn attention to a change in the mucus glands of the respiratory tree. He remarks that the glands in the trachea and bronchi are distended and the lumens filled with a thick material similar to that found in the pancreatic tissue. Exactly what is meant when he says it is similar to that found in the pancreas is difficult to say. The material in the mucus glands of the trachea stains like mucus, while that in the pancreas does not. There does not appear to be any lamellated material as in the pancreas. He believes that the mucus in the lumen of trachea and bronchi is unusually thick and because of that he believes there has been inspissation of secretion. Undoubtedly, in many cases, the mucus glands are dilated and filled with mucus, the nuclei of the cells are flattened against the basement membrane and the cell outlines are most indistinct. However, in some cases the mucus glands appear normal.⁷¹ Baggenstoss and Kennedy² state that in their cases the bronchi and their glands contained an exudate more purulent than mucoid and that only occasional glands were distended. Chronic infection of the trachea and bronchi cannot be ruled out as a cause of this histological change. Although no proof has been offered as yet that the secretion is altered, nevertheless, this is an important observation which requires further confirmation.

Liver. The liver is usually increased in size, is yellow in color and somewhat softer than normal. Andersen¹⁰ states that a fatty liver was found in 19 or about a third of her cases. Among these, 21% occurred in the age group of 2 weeks to 6 months and 60% in those dying after 6 months of age. Philipsborn, *et al.*⁵² reported 15 out of 26 cases as showing steatosis. All their patients without fatty

livers were under 9 months of age. In the few cases of Farber's^{24c} series in which there was no fatty metamorphosis of the liver, the pancreas showed only slight degrees of atrophy. He believes that a rough direct correlation could be made between the amount of fat in the liver and the degree of fibrocystic disease of the pancreas. Such fatty livers are found in experimental work on dogs, where complete depancreatization even with the use of insulin results in the liver becoming fatty. On the other hand, in dogs,³¹ monkeys,^{11a} cats,²⁹ and possibly humans,^{11a} where ducts have been ligated or removed with resultant atrophy of the pancreas, there has been no tendency to liver steatosis. The experimental work on the lipotropic substances (either the internal secretion of Dragstedt, or choline or lecithin) is too controversial for final evaluation. Dragstedt²¹ believes it might originate from the alpha cells of the pancreas, which would be supported by the fact that in pancreatic duct obstruction, fatty livers do not tend to be found as the islets of Langerhans are intact. Cole and Howe¹⁴ describe a case in an adult and have collected 5 from the literature in which there was atrophy of the pancreas (exact etiology uncertain), with marked steatosis of liver. These authors believe that both in the reported cases in adults and in fibrocystic disease of the pancreas in children, the fatty metamorphosis of the liver is secondary to the pancreatic disease. If this is so, one must look elsewhere than in islets for the source of lipocaine. The significance of the high incidence of fatty liver in fibrocystic disease requires investigation as to whether it is a general effect of poor nutrition or is specifically secondary to pancreatic disease.

Histologically, the hepatic cells contain large droplets of fat (fatty metamorphosis). This is uniformly distributed throughout the organ. In the earlier stages it may be most marked about the central vein. Andersen states that hemosiderin is present in the liver and Kupffer cells almost

invariably but Farber makes no mention of this, nor does any other author.

In a small number of cases (the exact incidence is unknown but Andersen^{1a} reports 4 cases) there is a cirrhosis of the liver centered about the portal areas. Grossly, there are large coarse and rather irregularly distributed scars in the liver, partly marking off areas of liver parenchyma of varying size. Farber^{24c} thinks the cirrhosis is due to intrahepatic biliary obstruction as he found the small bile ducts dilated and filled with eosinophilic material like that in the pancreas. He considers it likely that this secretion consists of mucoprotein and its presence, as in the pancreas, leads to obstruction and dilatation of the ducts with atrophy of the parenchyma drained by these ducts, leading to condensation of the stroma, and development of a somewhat focal type of fibrosis. It appears to be a biliary type of cirrhosis but jaundice has never been reported. Proliferation of bile ducts has been observed.⁷¹ Initially the cirrhosis seems to be focal in distribution and even in advanced cases, normal portal areas may be seen.

Additional Findings. Grossly in 8 cases of Andersen's^{1a} series the *gall bladder* was small and contained a small amount of translucent gray mucus and in 4 of these cases the cystic duct was atretic, but in most instances this organ and the bile passages are not reported upon. Andersen had 1 case with atresia of the bile duct, atrophy of the gall bladder and cysts of the cystic duct and gall bladder. Six or 30% of her own cases presented evidence of cystic duct atresia. No abnormalities of the common or hepatic ducts have been reported except in 1 case⁴⁹ which showed narrowed common and hepatic ducts. Farber mentions that frequently the gall bladder mucus glands are distended and in this respect resemble the glands of the pancreas. Furthermore, he found the gall bladder to be often small and its contents thicker and stickier than normal.

The *spleen* is normal or slightly enlarged. In 1 case with cirrhosis of the liver the

spleen was large and somewhat fibrous.³⁵

Changes in the *alimentary tract* are not commonly mentioned but dilatation and hypertrophy of the colon have been recorded.^{1a,13} Microscopically a moderate infiltration of lymphocytes and plasma cells with occasional polymorphonuclear leukocytes and fibrosis of the submucosa of the bowel were seen in a few cases.^{13,33} Two of Andersen's cases had atresia of the small bowel and 1 had an obstructing band across the ileum. All 3 died at 4 to 6 days. Farber^{24c} mentions dilatation of mucus glands of the duodenum, the jejunum and the esophagus with thick material as in the pancreas. Baggenstoss and Kennedy² could only find dilatation of the mucus glands of the duodenum.

The *heart* is usually normal although it may show fatty infiltration. Cor pulmonale without histological changes in the pulmonary vascular tree has been noted in 1 case of fibrocystic disease with bronchiectasis.²² The incidence of congenital heart disease is not increased in fibrocystic disease.

In those cases with severe vitamin A deficiency, keratinizing metaplasia may be found in the salivary glands, larynx, trachea, bronchi, uterine mucosa, periurethral glands, pelvic mucosa of the kidney, ureters, bladder, etc. Farber^{24c} reports dilatation of the acini of the sublingual, submaxillary and parotid glands by inspissated secretion. Wilson and Dubois⁷² reported a marked inflammatory infiltration of salivary glands but found only an occasional dilated acinus. Oppenheimer⁴⁸ likewise reported the finding of only a few dilated acini in the salivary glands. Inclusion bodies have been reported in salivary glands of a few cases.^{9,72}

The *reproductive organs* are rarely commented on but Flax, *et al.*²⁶ report a case of probable meconium ileus in which the uterus and vagina were large and there was a profuse mucus exudate in the cavities of both. Sections of the uterus showed large distended mucus glands in place of the normal endometrium. Atresia of the ureters is not uncommon.^{1a} Calcium salts

in the cells of the kidney tubules has been reported.² Sections of the skin and subcutaneous tissues show almost complete absence of fat.²⁷

Meconium Ileus. Meconium ileus^{10,19,-20,21d,36,39,41,42,66} is a clinical term for a condition found in the newborn which is intimately associated with lesions of the pancreas identical with those found in older infants and children and called cystic fibrosis of the pancreas. It is characterized by intestinal obstruction in the newborn as a result of inspissation of the meconium which becomes so thick and sticky as to be immobilized in the gut.

This was first described by Landsteiner¹² in 1905—who ascribed it to lack of external pancreatic secretion resulting in failure of liquefaction of the meconium. This hypothesis seems to have been confirmed by Farber.^{21d} The causation is either: (1) congenital stenosis or atresia of pancreatic ducts^{36,41} or (2) acinar and duct obstruction in inspissated secretions^{21c,21d} or (3) possible failure of normal development of the pancreas.³⁹

In any case, the histological findings in the pancreas are identical with cystic fibrosis. Since at the most these patients do not live beyond a few weeks, there is as yet no evidence that they would develop the lung lesions or lesions of other secreting glands as in the older group. Therefore it is unknown whether the pancreas is the only organ involved.^{21c} If Farber's hypothesis, regarding fibrocystic disease of the pancreas as being a systemic disease, is correct, one should in some of these cases expect the glandular changes to be present elsewhere in the body, but as yet there has been no note of them.

The 3 adequately reported cases of meconium ileus,^{56,41,42} in which the duct of Wirsung was examined in serial microscopic sections, showed marked stenosis of the ducts, a short distance from the ampulla of Vater. The ducts, distal to the stenosis, were dilated and Hurwitt and Arnheim⁵⁶ point out that primary parenchymal fibrosis does not produce such dilatation of the distal parts of ducts.

They regard these stenotic ducts as being due to a focal developmental defect and not to failure of the pancreatic ducts to recanalize in embryonic life. They suggest that the pancreatic lesion is an early intra-uterine lesion, the time of origin of which might be roughly estimated by the distance the meconium has traveled. In their own case the meconium was at the ileocolic junction and in Kornblith and Otani's case at the hepatic flexure. They quote Keith⁴⁰ as stating that meconium reaches the ileocolic junction by the fourth month and the rectum by the fifth month of fetal life.

In Kaufman and Chamberlin's³⁹ case the ducts were not examined but in extensive sectioning of the pancreas they failed to find more than one fully developed excretory duct. No small ducts with normal tall columnar epithelium were seen. The structure of the acini was not normal and their impression was that the development of the acini was faulty and that maturation of small ducts into acini had been prematurely arrested.

BIOCHEMICAL DATA. Chemical analysis of the stools has always held an important rôle in the investigation of the celiac syndrome and in experimental work with animals. It would now appear, however, that their diagnostic rôle will be less important in the future and will be replaced to a large extent by estimation of the duodenal enzymes. Nevertheless, the estimation of fat, protein and carbohydrate in the stools has played an important part in the understanding of pancreatic deficiency.

It may be briefly pointed out that large amounts of the fat, protein and carbohydrate in the diet fail to be absorbed and are lost in the stool, particularly in the case of the former two. The loss of protein is particularly important and accounts, at least in part, for the failure of development and the emaciation in the subjects of this disease. The many factors that influence the loss of these substances, and the care that must be taken in their quantitative estimation and the interpreta-

tion of the latter may be found in the references in the bibliography.^{1a,1d,1e,31,37,52,63}

The loss of fat, protein and carbohydrate in the stools of cases of fibrocystic disease of the pancreas is similar to that found in experimental animals in which the pancreatic ducts are ligated.^{29,31} Attention is drawn to the fact that effects of ligation vary considerably from time to time in the same animal and from animal to animal,^{31,37} and probably the same applies to cases of fibrocystic disease, accounting for variations in the stools and in symptoms. The processes of digestion and absorption are complicated and there are still too many unknowns, to lay down hard and fast rules. However, it may reasonably be concluded from the stool changes in fibrocystic disease and their correlation with experimental work, that the exocrine pancreatic juices are lacking.

The estimation of the duodenal enzymes has assumed great importance since the separation of fibrocystic disease of the pancreas as a disease entity. Much investigation^{1c,25,44,62,73b} has been directed towards this problem and as the result of advances in technique, clinical interest has been regained in the quantitative estimations of the enzymes. Their estimation in fibrocystic disease is essential for clinical diagnosis.

In fibrocystic disease the values are very low or zero for all enzymes, well below the low limits of normal cases or of other diseases. Estimation of trypsin only is necessary. The low values persist even on repeated examinations.²⁵ It is important to note that the duodenal fluid is scanty and very viscous as might be expected from the histological appearance of the secretion in the pancreas. Intravenous secretin fails to affect the volume of the fluid or its enzymatic content,^{44,53} except possibly in early cases.

The results of enzyme estimations confirm the histological appearance of marked pancreatic dysfunction. It is not known whether the pancreas fails to produce enzymes or whether the escape of these is

prevented by increased viscosity of the secretions. As some cases have a small amount of enzymes present, it may be that enzymatic secretion is normal but the secretions cannot escape. On the other hand their presence might be accounted for by areas of normally functioning tissue, such as are sometimes seen histologically. Much more correlation between enzyme activity and histological findings is required before any conclusion can be reached.

As regards gastric analysis little or no information can be found in the literature but Andersen (personal communication)¹⁴ states that in general, except in extreme marasmus, the acidity and rennin content of the gastric juices are normal. This is a rather important observation from the viewpoint of Farber's theory that this disease may be a general one affecting secreting glands. The stomach would then have to be excluded from the general thesis.

In general, chemical examination of the blood is not of great value. The glucose tolerance curve may be flat^{1b} or high.^{32b} Blood cholesterol tends to be low.^{1a,1b} Total lipids and their fractions are essentially normal.⁴³ The fasting vitamin A content of the serum is low⁵² and the vitamin A absorption curve is flat.⁶ These findings seem to confirm the failure of adequate absorption, particularly of fat, but throw no light on the etiology of the disease.

ETIOLOGY AND PATHOGENESIS. Before commencing to discuss these aspects of the disease, it is well to remember that the pancreas is but one supply depot of enzymes in the complicated and lengthy process of digestion, commencing in the mouth and ending with the expulsion of the undigested residue. In addition to the digestion of food, there is the equally complex process of absorption. One or both mechanisms may be affected and to distinguish the rôle of each in disease, is difficult. Experimental work has defined areas in the gut where certain processes are known mainly to take place but their

correlation with other parts and their ability to take over functions, one from another, is not clearly defined. For years arguments have been going on as to the importance and necessity of the exocrine secretions of the pancreas in digestion. So far as dogs, rabbits and cats are concerned, it is apparent that the exclusion of the pancreatic juices does affect their health, although not in a sharply defined manner. Much care must be taken to exclude all pancreatic juices from the intestine, for functional disturbances can be prevented if only 1 cm. of pancreas is left attached to a patent duct.⁵⁶

The importance of the pancreatic enzymes in digestion has recently been again clouded by the results of surgical exclusion of the pancreatic juices in humans.^{11a} A majority of the cases in a small series did not develop symptoms of pancreatic dysfunction. Brunschwig,^{11a,11b} after transecting the head of the pancreas and ligating the ducts in monkeys, has found that they live and thrive for several years without evidence of disturbed digestion. There was no evidence at autopsy that the pancreas was functioning.

As a result, in any disease which shows marked pancreatic change, one must be careful in attributing a host of symptoms, signs and biochemical findings entirely to dysfunction of this organ. Therefore, in fibrocystic disease of the pancreas, although many of the celiac symptoms are attributable to deficiency of the pancreatic secretion, one must be wary of assuming that this disease entity is dependent wholly on the pancreas. Such an assumption may be later proven to have been wrong. In short, the pancreas may be only the symbol of a much more generalized disease, as pointed out by Farber.^{24c}

Nevertheless, all discussions of etiology and pathogenesis center on attempts to explain the changes in the pancreas. There is general unanimity in regarding the pancreatic lesions as of obstructive origin,^{1a,24c} but the exact mechanism of obstruction is uncertain. The very nature of the lesion with its dilated acini and

ducts filled with secretion and the ultimate atrophy and replacement fibrosis, indicates obstruction. The end results are similar to those following ligation of the ducts experimentally and in cases of obstructed ducts.^{4,47} Other than the final result, it is questionable whether the changes following sudden complete obstructions of the pancreatic ducts are comparable to the obstruction in fibrocystic disease. In the latter disease the obstruction is probably partial at first and slowly progressive, possibly never to become complete.

The etiological theories, regarding the mechanism of obstruction, can be discussed under three headings: (1) Infection, (2) Vitamin A deficiency, (3) Congenital defect: (a) Obstruction from atresia, (b) Obstruction from production of an abnormal secretion.

Infection. It has been suggested^{1a} that the presence of minor degrees of inflammation in the pancreas might indicate a fetal or post-natal infection. The main evidence against infection is the absence of a known infecting agent; the failure to find inflammation of the pancreas in the presence of gastro-intestinal infections; the absence of chronic biliary infection in children and the presence of similar inflammatory infiltrations in the degenerating pancreas in experimental ligation of the ducts. The familial incidence is also against fetal inflammation. Congenital lues is ruled out by the failure to find evidence of syphilis in the majority of cases. Although a few cases show inclusion bodies in some of the tissues,⁹ the incidence is no higher than that found in routine autopsies.^{24c} Finally, inflammation of the pancreas does not explain the high incidence of lung lesions in this disease.

Vitamin A Deficiency. The chief difficulty in assigning the causation of fibrocystic disease to this deficiency is the absence of squamous metaplasia of the pancreatic ducts of such extent as to cause obstruction, except in rare cases. Extreme cases of vitamin A deficiency may show no pancreatic change.⁷ There

is no evidence that pre-natal deficiency of vitamin A is a causative factor.^{1a}

The balance of evidence suggests that vitamin A deficiency is a complication and not the cause of the disease. However, this deficiency, with considerable reason, has been regarded as the cause of the lung lesions.^{1a,49} Here again, although the relationship has not been clarified, the weight of evidence appears to be against vitamin A deficiency as a primary cause of the pulmonary infection.^{24c} Lung infection is not common in idiopathic celiac disease in which vitamin A absorption is poor, or in adult humans or animals with pancreatic duct obstruction. On the other hand, Oppenheimer's patient with straight-forward duct atresia died with pulmonary disease, which may have been secondary to the vitamin A deficiency present. Until direct evidence is found, the relationship of the pulmonary lesions to vitamin A deficiency will remain obscure.

Congenital Defect. The fact that the lesions are frequently present at birth or develop in early infancy, as well as the familial incidence and the frequent presence of atretic ducts elsewhere, suggest strongly that the basic abnormality is a congenital one. Yet, it differs from most congenital anomalies, such as atresia of the bile ducts or congenital heart disease, in the absence in most cases of a gross anatomical defect. The variation in the severity of the microscopic lesions and the survival of a considerable number of patients well on into childhood is puzzling and one must assume that the basic defect continues to operate with varying intensity long after birth and in a different manner from a clear-cut anatomical defect. For these reasons, the reviewer believes it important in fibrocystic disease to separate those cases secondary to congenital atresia of the ducts from those without atresia. There seems to be no reason why pancreatic duct atresia could not occur as an isolated anatomical defect, in which case the secreting glandular structures of the bronchi and other epithelial tissues would not necessarily be congenitally abnormal.

On the other hand, in those cases without atresia, it would be reasonable to hypothesize, as Farber has done,^{24c} that there is a generalized defect of the exocrine glandular secreting tissues, including the pancreas, if the anatomical evidence supported the theory.

(a) *Congenital Duct Atresia.* There seems to be no doubt whatsoever, that a few cases of fibrocystic disease of the pancreas, excluding cases of meconium ileus, have their origin in atresia of the pancreatic ducts.^{5,48} Although Andersen^{1a} and Farber^{24c} state that congenital atresia of the main ducts is uncommon (except in meconium ileus), this is by no means certain as most of the examinations of the ducts appear to have been made by gross dissection and in but few cases have microscopic serial sections been made. The difficulty of tracing these ducts grossly^{1a} in infants emphasizes the necessity of careful serial microscopic examination of these ducts. Furthermore, the head of the pancreas and second part of the duodenum should be removed *in toto*, routinely at autopsy, and kept until sections of the pancreas are examined. Andersen has suggested^{1a} that when stenosis or atresia is present it may have been the result rather than the cause of the fibrosis.

A theory which comes under the heading of atresia is that proposed by Rauch, *et al.*⁵⁹ This is based on the common origin of the anlagen of the lung and pancreas from the entodermal tube. In order to bring the pancreatic and lung lesions into relationship, they proposed that at some stage of development of these organs, as a result of budding, a constriction of some of the excretory ducts of both organs might occur, giving rise to congenital bronchiectasis and congenital fibrocystic disease. Although some pancreases show apparent failure of proper development,²⁹ no factual proof for this theory has been adduced.

Another form of obstructive lesion described by Baló and Ballón³ (not necessarily congenital), consisting of focal epi-

thelial metaplasia of the ducts of the pancreas, causing small duct obstruction, is ruled out by their absence in the 5 cases examined by Rich and Duff.⁶⁰

(b) *Theory of Abnormal Secretion.* This reasonable, intriguing and all embracing hypothesis has been developed by Farber^{21c} in an attempt to explain particularly the association of lung and pancreatic disease and to account for the pancreatic lesions in the absence of main duct atresia. In brief, he believes it is a systemic disease, of which the pancreatic changes are only a part, although they may predominate. It is based on a statement by Blackfan and Wolbach⁷ that the pathogenesis of fibrocystic disease of the pancreas lay in the production of an abnormal secretion, but Farber has extended the theory to include other epithelial lined organs. His study of 87 advanced cases and of about 350 early cases^{24b} leads him to believe that the basic lesion is a physically altered secretion causing obstruction of acini and small ducts, later involving large ducts and leading to atrophy and fibrosis. The definite absence of duct atresia which has been proven in a few cases,^{1a,2,49,61} the absence of any large duct dilatation in some cases^{21c} and the thick scanty duodenal juices, is evidence in favor of an abnormal pancreatic secretion. Furthermore, the presence of aberrant pancreatic tissue showing similar changes to the pancreas, in 3 cases,^{1a,24c,68} argues that intrinsic biochemical alterations of the secretion are fundamental. Unfortunately there is no direct proof of this alteration nor evidence as to its nature.

The evidence for altered secretion in other epithelial lined organs is less convincing. Farber^{24b,24c} claims that many cases show dilatation of glands and inspissation of secretion in the trachea, bronchi, esophagus, duodenum, gall bladder, salivary glands and jejunum. All cases reported previous to his publication make almost no mention of such findings, although in 2 cases^{45,72} the salivary glands showed minimal dilatation of acini. As has been previously indicated, the one study re-

ported² since then was not strikingly confirmatory. It is apparent that confirmation of the extra-pancreatic lesions is still lacking, although it may be noted that the liver lesions of the associated cirrhosis bear a striking resemblance to those of the pancreas.⁷¹

As further proof of his theory, Farber^{24c} has reported a case in which the lungs and liver showed the above-mentioned changes in a patient who had no symptoms referable to pancreatic achylia and in whom at autopsy a normal pancreas was found.

Farber has suggested^{21c} that the character of the duodenal drainage and the inspissated secretions in the acini are reminiscent of vagal stimulation (as has been known for years vagus stimulation produces a thick secretion, high in enzyme content). He suggests that a disturbance of parasympathetic innervation or an autonomic imbalance in nervous control of secretions in the pancreas and in mucus glands may be at fault. He has produced^{21a} a similar lesion in the pancreas of kittens by the injection of a parasympathomimetic drug (pilocarpine) over a period of 2 to 8 weeks. Extreme loss of weight and severe nutritional disturbances were the chief clinical features. As far as the reviewer can find, no one has repeated these most interesting experiments.

Summary. Fibrocystic disease of the pancreas is the name given to a disease, whose etiology is completely unknown, because the pancreas shows the most striking histological changes. In the absence of a better name, one must remember not to place too much emphasis on the pancreatic changes until it is proven to be essentially a disease of the pancreas alone, or otherwise. In view of the contradictory experimental findings in animals and humans where exocrine secretion of the pancreas has been excluded from the gut, one must proceed most cautiously in assigning to the pancreatic lesions in this disease an all-important rôle.

Clinically, the disease is characterized by severe respiratory infection and gastro-

intestinal symptoms and signs of the celiac syndrome, the former tending to predominate under 1 year of age and the latter over 1 year of age. In the neonatal period it gives rise to the clinical entity called meconium ileus. The disease appears to be congenital and not infrequently familial.

Pathologically, the essential lesion in the pancreas is obstruction and dilatation of acini and ducts by an inspissated secretion with secondary atrophy and fibrosis. Associated with this is the almost constant finding (except in very young infants) of chronic respiratory infection with a mild bronchiectasis, abscess formation in the lungs and bronchopneumonia. The liver shows marked fatty metamorphosis in about a third of the cases and in a small percentage biliary cirrhosis of a somewhat focal nature is present. In meconium ileus the pancreatic lesions are present and the bowel is obstructed by a thick mucilaginous meconium which is formed in the absence of pancreatic juices.

Several etiological factors have been put forward, of which only two can reasonably be considered in the light of our present knowledge. The first is the theory of congenital atresia which postulates that there is a congenital atresia or stenosis of the pancreatic ducts, or failure of normal development of the pancreas. Atresia is more common in meconium ileus and unquestionably accounts for a certain number of cases in older children. With more detailed and adequate examination of the duct system, a definite number of cases will undoubtedly be isolated from

the group and placed in their proper etiological relationship. However, a larger proportion of cases appear (and one uses this word advisedly) to be due to an intrinsic obstruction, probably commencing in the acinar area which may be due to an unknown congenital defect in the function of the pancreatic acinar cells themselves, resulting in an unusually thick secretion. This theory, developed by Farber, is most important but lacks adequate proof. He believes, in addition, that there is a widespread involvement of variable degree, of other mucus secreting structures in the body as shown by the change in the physical character of the mucus produced. There may be either a deficiency or insufficiency of mucinase required for the maintenance of secretions in the normal state. The type of secretion present is reminiscent of the results of vagal stimulation. Neither Farber's histological findings nor his experimental work have as yet been confirmed.

The possibilities of further studies on fibrocystic disease of the pancreas are almost unlimited. Detailed and careful pathologic studies alone would be useful in clarifying many obscure points, while there is a wide scope for experimental work in the pathologic physiology. Finally, it may be stated that in spite of the spate of literature in the last 8 years, the great progress in diagnosis, and the slight progress in treatment of this disease, the etiology is not known and in particular the relationship of the pancreatic and lung lesions has not been elucidated.

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PREVENTIVE MEDICINE AND EPIDEMIOLOGY

UNDER THE CHARGE OF

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PROFESSOR OF PREVENTIVE MEDICINE AND EPIDEMIOLOGY, HARVARD MEDICAL SCHOOL
BOSTON, MASSACHUSETTSMATERNAL DISEASE AS A PRINCIPLE IN THE EPIDEMIOLOGY
OF CONGENITAL ANOMALIES

WITH A REVIEW OF RUBELLA

BY W. LLOYD AYCOCK, M.D.

AND

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Harvard Medical School and School of Public Health; and the Children's and Infants'
Hospitals, Boston, Massachusetts.)

Congenital Anomalies Following Ru- cataract were observed in Sydney. When
bella. In the early months of 1941,* an they continued to appear, in numbers
unusual number of cases of congenital indicative of no chance phenomenon, seri-

* In 1940 the Medical Journal of Edinburgh carried an editorial, not to call attention to anything new about rubella, but only to celebrate the 100th anniversary of the appearance in "The Journal" of the first description of the disease "in our language."¹² The editorial opens, "German measles has been in the news of late, and we hear of medical officers of health, headmasters, headmistresses, and others clamoring to have it deprived of . . . its place as a notifiable disease and relegated to the limbo of minor exanthemata . . . a nuisance rather than an illness." The identity of "Rötheln" had already been established in the German literature and Dr. Robert Patterson of the City of Leith, being convinced from many cases "which lately prevailed in this neighborhood that it possesses characters peculiarly its own," was induced to present a comparison with measles and scarlet fever in tabular form, and the clinical aspects of the disease quaintly captured in his phrase that "the little patients are observed to draw nearer the fire." The editorial concludes that "little seems to have been added in the intervening hundred years," a statement borne out by reference to almost any text written up to within very recent years where the disease is quickly dismissed as of no consequence in itself and without complications or sequelæ.

If "little seems to have been added in the intervening hundred years," the editorial commemorating the centenary of Patterson's description of the disease set the stage, as it were, for events to come within the next few months, in the first year of the second century, which dramatically removed rubella from the "limbo of minor exanthemata." Its role as a "nuisance rather than an illness" was unmasked by a group of Australian investigators whose clinical acumen and epidemiological insight no doubt would be applauded by Patterson himself, and will be celebrated in 2041 by another editorial in the illustrious Journal.

Rubella now takes its place as one of that group of diseases where infection in the many is trivial, but where sequelæ in the few constitute major problems of epidemiology and preventive medicine. As if to outdo its fellows, and to elude medical science, rubella is so mild and its sequelæ so severe that any connection between them would hardly be suspected. To cover its tracks, unlike poliomyelitis with its paralysis on the third day and the mastoid or pneumonia on the heels of the rash in measles, it put so much distance between itself and its sequel—at least several months, and into the next generation—that it was not detected until with epidemiologic overconfidence from a long period of quietly "getting away with it," so to speak, it tried to make up for lost time with its "virgin soil" outbreak in Australia. This circumstance related to us by Burnet on his visit to this country in 1944,³ is summarized by Scholes as follows: "During the past 35 years the first large epidemic of rubella occurred in 1914, the next in 1923 and the last in 1937. The 1937 outbreak spread to all states, but had not died out in remote districts when war broke out in 1939. In that year, with the mass movements of young people from state to state and from country districts, the disease became epidemic, sweeping through the whole country and waves had recurred until fairly recently. Between 1923 and 1937 there was no opportunity for anyone to contract rubella and from 1937 to 1942 there was every opportunity, the vast majority of young adults and women of child-bearing age being susceptible."¹³

Finally, as if to shield itself from medical intrusion, rubella selected as its channel of operation a condition least likely to be interfered with in the interest of prevention, for pregnancy is so dominated by religious, moral, emotional and legal considerations, that its purely medical aspects may be secondary to them. Indeed at times they have been taken into consideration only insofar as they afforded a basis for some action dictated by these other attitudes. The Pilgrims, for example, whose major epidemiologic pursuit seems to have been the search for sin, and who believed the period of gestation was 9 months to the day, sent many a young couple to the stocks "for having a child before the natural time of woman after marriage."

ous thought was given to their causation.^{19a,19b,19c} Their almost epidemic occurrence, their ophthalmologic characteristics and their association with congenital heart disease suggested to Gregg that the etiology was a constitutional, toxic, or infectious disturbance in the fetus, rather than a purely developmental defect. Calculations relative to the stage of pregnancy, when the congenital anomalies must have originated, showed that it coincided with the period of maximum intensity of a widespread epidemic of rubella. Gregg collected 78 instances of congenital defects, the basis for which was found to lie in maternal rubella during pregnancy. His reports were confirmed and the investigation extended by Swan and co-workers.^{41a,41b}

TYPES OF CONGENITAL ANOMALIES. The special ophthalmologic features were cataract, usually bilateral, and of a subtotal type associated with nystagmus and a sluggish pupillary reaction to light; in some cases, an atrophic appearance of the iris; and rarely microphthalmia and buphthalmia.

On histologic examination, Swan⁴⁰ found the peripheral lens fibers showed evidence of degeneration or necrosis, and were separated by vacuoles, while the central core was amorphous in appearance.

The cardiac manifestations were a harsh systolic murmur over the precordium, usually loudest over the base of the heart or pulmonic area, and absence of cyanosis. In each of 3 afflicted infants autopsied by Swan,⁴⁰ the ductus arteriosus was widely patent, there was variable patency of the

foramen ovale, and 1 baby had an interventricular septal defect. As regards malformations of the heart Swan quotes Bedford and Brown³ who state that "the critical period for the development of congenital cardiac defects is from the fifth to the eighth week of intrauterine life, during which the septa are forming, the bulbus cordis is undergoing involution, and torsion of the great vessels is taking place." Subsequently, other forms of congenital heart disease accompanied by cyanosis have been reported.

Green and Dogramaci¹⁸ who reviewed the records of 434 children studied at the Children's Hospital, Boston, for congenital heart disease, found a history of maternal rubella in 4 instances and exposure of a susceptible mother in 1 instance. They presented data as shown in Table 1.

Assuming that there had been 100% accuracy in diagnosis and reporting of the disease (in the hospital records), the inference would be that rubella was the etiologic agent in approximately 1% of all types of congenital heart disease. It is reasonable to conclude that reliance on hospital records alone would result in the loss of many cases in which the infection had been regarded as trivial, forgotten or merely overlooked, and that the true figure will eventually be shown to be substantially higher. That this will prove to be the case, is also suggested by the work of Conte and co-workers⁸ who surveyed the records of all congenital anomalies at the Vanderbilt University Hospital. Of 120 anomalies there were 4 cases of congenital heart disease in which maternal

TABLE 1.—DATA IN 5 CASES OF CONGENITAL HEART DISEASE RELATED TO RUBELLA (GREEN AND DOGRAMACI¹⁸)

Stage of pregnancy when rubella occurred	Presence of cataracts	Nature of cardiac defect	Physical signs	Roentgenological findings
1 month	+	Tetralogy of Fallot	Typical, cyanosis	
3 months	+	Uncertain	Rough systolic murmur over precordium	"Globular heart"
2½ months	+	I.V. septal defect	Harsh to and fro murmurs in pulmonic area, with grade 2 murmur over apex	"Globular heart with broad base"
1 month	0	Patent ductus arterio-us	"Typical"	Typical
2 months*	0	Pulmonary stenosis; I.V. septal defect	Cyanosis	

* Exposure to rubella.

rubella appeared to be the inciting agent. The incidence in this series would be around 4%, since other anomalies as well as heart disease are included. The higher figure is to be attributed to additional information obtained by a questionnaire mailed direct to all mothers.

In addition to the malformations enumerated above, deaf-mutism has been reported by Swan and his co-workers^{41a,41b} in a total of 12 out of 43 children born to mothers who suffered from rubella during pregnancy. Some form of deafness was noted by Carruthers⁶ in 74 out of 102 such cases. Muteness when present was regarded as secondary to deafness. In an autopsied case, the outstanding defect was the total absence in both ears of any differentiation of the primitive cells to form the organ of Corti.⁴⁰

Evans¹⁵ found that of 34 babies whose mothers suffered during pregnancy from rubella, 23 exhibited congenital dental abnormalities, in 18 cases major in nature, such as "sharklike" and "pointed" incisors and enamel hypoplasias. All except 2 of the infants showed other congenital malformations.

Finally, it was noted that most of the babies were of small size, ill-nourished, and difficult to feed. No less than 15 of the 78 patients studied by the Australian workers were dead by the end of 1943, and many of those living showed mental as well as physical retardation.

These reports have been confirmed in this country by Reese,³⁶ Rones,³⁷ Erickson,⁴¹ and Albaugh,¹ who add 18 more cases to the literature.

STAGE OF PREGNANCY. The combined work of the authors cited indicates that the average period of the pregnancy when rubella occurred was as follows:

For cataract	6 weeks
For deafness	9 weeks
For cardiac anomalies	5-10 weeks
For deformed teeth	6-9 weeks

Carruthers, however, felt that although the average date was of some significance, "the importance of the stage of pregnancy at which infection occurs is that, if it is

in the first 6 weeks, fetal damage may be widespread and include the eyes, both divisions of the ears, the heart, and perhaps many other parts. After the sixth week, the eyes may escape, the heart may be spared, and the semi-circular canals may become normally developed; but the cochlea is still likely to be damaged." No correlation could be established between the clinical intensity of the maternal infection and residual fetal damage. There would be no reason for doubting that the disease in question was rubella, or that the syndromes produced were new, except as they were newly recognized. Chandler⁷ is of the opinion, however, that the resulting type of congenital cataract is seen more frequently now than formerly. It is not known to what extent this increase may be accounted for by the better practice of obstetrics and pediatrics which now bring to term more pregnancies which result in a living baby. This seems to be the case in retrolental fibroplasia, described by Terry.⁴³

FREQUENCY OF ANOMALIES. Most of the earlier studies have started with cases of congenital defects of the sort described. The finding of maternal rubella in a high percentage of the cases has been interpreted by some as indicating that the defects always follow the infection. For example, it has been stated that "available data would suggest that 100% of the mothers who contract rubella in the first 2 months, and approximately 50% of those who contract it during the third month, will give birth to infants with congenital anomalies."¹ As pointed out by Albaugh, many questions have been raised which will require for their answers extensive epidemiologic studies. An editorial in the *Lancet* in February, 1946,¹³ states that "It is four years since Gregg in Sydney reported that congenital malformations . . . were occurring in infants whose mothers had had German measles early in pregnancy . . . yet nobody seems to have taken the few simple steps needed to find out whether rubella is a serious cause of congenital

defects . . .” The only study which represents an adequate attempt to determine the numerical probability of congenital anomalies following rubella during pregnancy is that of Fox and Bortin.¹⁷ They found that of 152 married women who contracted rubella during 1942, 1943 and 1944, 11 had rubella during pregnancy: 5 during the first 2 months, 4 during the second to the fourth month, 1 in the seventh, and 1 in the ninth month. They found only 1 of 11 cases (rubella in the first month of pregnancy) which “evidenced a pathologic course.” The child in this case was stillborn. However, they present a second instance (rubella in the second month of pregnancy) in which the outcome was a “blue baby, sinus disease, otitis media, hydrocephalus (which receded spontaneously), perfectly normal at present.”

Approaching the problem by the same method, we have investigated 1300 cases reported to Boards of Health of 2 Massachusetts communities for instances of rubella in pregnancy, and have found 4 as follows:

Case	Length of pregnancy before rubella	Result
1	2d month	normal baby
2	2d month	mental retardation
3	4th month	normal baby
4	9th month (12 days antepartem)	normal baby

If these 4 cases are added to the 11 cases reported by Fox and Bortin, there are 15 instances of rubella in pregnancy where data on the outcome are available. The disease occurred in the first 3 months of pregnancy in 11, with 8 normal and 3 abnormal babies resulting: 1 infant with spontaneously subsiding hydrocephalus, 1 mentally retarded child, 1 stillborn infant, and 8 normal infants. Four cases occurring from the fourth to ninth month all resulted in normal babies.

In this small series, the morbidity in the baby is 27 %, when the disease occurred in the first 3 months of pregnancy.

That a study based on cases reported to Health Departments may represent an incomplete picture of this phase of the problem, may be seen from the following cases found in the course of this study, in only one of which had the rubella in the mother been reported.

To these 5 can be added 2 of our cases in which spontaneous abortion resulted.

Thus, on the basis of admittedly incomplete data, it would appear that the risk of anomalies in the infant may be upwards of 25 % following rubella during the first 3 months of pregnancy. Whatever the final incidence turns out to be, it will be sufficiently high as to constitute a problem of a very serious nature to the parents concerned and their obstetrician. Available data do indicate that the first 3 months of gestation is by far the most vulnerable period. Of 100 cases reported by Carruthers, Swan, Erickson, Reese, Rones, Green, Albaugh and Kraus,²⁹ 94 % of the mothers were recorded as having suffered the infection during the first 3 months (Table 3).

ABORTIONS AND STILLBIRTHS. Nowhere in the literature, except for Fox’s mention of a stillborn baby following maternal rubella, have we found mention of the possibility that death of the fetus and spontaneous abortion may result. That this may occur is suggested by 4 cases of which we have personal knowledge (Table 4).

Congenital Anomalies and Other Maternal Diseases. No studies have appeared with respect to congenital defects following other maternal infections, but 3 instances of such defects, 1 following chicken pox, 1 poliomyelitis, and 1 influenza, have recently been observed in Massachusetts.

Case	Length of pregnancy before rubella	Defect	Reported to Board of Health
1	2 months	Deafness	No
2	2 months	Mental retard	No
3	2 months	Heart disease, cleft palate	No
4	1 month	Heart disease	Yes
5	2 months	Cataract, heart disease, deafness	No

Mann³² has expressed the opinion that anomalies may follow other infections, and Swan⁴⁰ has postulated that more severe virus infections, such as rubeola, may kill the fetus, whereas the milder rubella merely damages the surviving fetus. Lynch³¹ has called attention to the high mortality of the fetus in maternal measles and smallpox.

The established relationship between maternal rubella and congenital anomalies, together with these observations of anomalies following other maternal illnesses, raises the question to what extent maternal disease in general may represent a principle in the etiology of congenital anomalies—heredity, for example, being another.

POLIOMYELITIS. In the course of a study of poliomyelitis in pregnancy from other points of view²⁵ records have been collected concerning 264 cases. Of these, some statement concerning the infant is given in 131. In Table 2 the outcome of the pregnancy is shown according to the

month of pregnancy during which the mother contracted poliomyelitis. Cases where the mother died of the disease with the fetus *in utero* are not included. Pregnancy terminated or resulted in a dead fetus in the 2 cases in the first month, in the majority of cases in the second month, and in 5 of the 10 cases in the third month. Thereafter the outcome was, as a rule, a normal child if the report "normal delivery" can be so interpreted. In 3 instances (second, third and seventh months) there were defects in the infants while in 3 others (ninth month) the infant was reported to have suffered from poliomyelitis. The evidence suggests that the risk to the fetus in maternal poliomyelitis is, as in German measles, high if the disease occurs in the first 3 months of pregnancy and less in later months. It is not clear, however, to what extent the hazard to the fetus may be the result of paralysis in the mother.

MONGOLISM. Ingalls and Davies²⁷ are

TABLE 2.—CONGENITAL DEFECTS FOLLOWING MATERNAL RUBELLA BY MONTH OF PREGNANCY

	1	2	3	4	5	6	7	8	9
Carruthers ⁶	2	8	6	3		1			
Swan ^{40a}	12	19	10	2					
Erickson ¹⁴	7	4							
Recse ²⁶	3								
Rones ³⁷	1	1	2						
Green and Dogramaci ¹³	2	2	1						
Albaugh ¹	2	4	3						
Kraus	1	4							
	30	42	22	5		1			

TABLE 3.—POLIOMYELITIS IN PREGNANCY: OUTCOME WITH REFERENCE TO INFANT BY MONTH OF PREGNANCY

Month of pregnancy	1	2	3	4	5	6	7	8	9	
Total cases	2	9	16	12	16	19	13	13	31	131
Normal child		1	6	4	7	8	5	8	15	
"Normal delivery"		1	4	6	3	6	2	2	11	
Cæarian section					1		1	1		
Total	0	2	10	10	11	14	8	11	26	92
Stillborn or died shortly	1	1	3	2	5	5	4	2	2	
Therapeutic abortion		1								
Abortion	1	4	1							
Miscarriage			1							
Total	2	6	5	2	5	5	4	2	2	33
Polio in baby									3	
"Lame"							1			
Congenital defect*		1	1							
Total		1	1				1		3	6

* 1 bilateral clubfoot, "not polio;" 1 congenital heart.

collecting clinical data on prenatal disease in relation to mongolism. A preliminary survey reveals the following 5 instances of intercurrent disease among 50 mothers who subsequently gave birth to a mongolian idiot.

Intercurrent disease	Stage of pregnancy
"Flu"	2 months
Rubella	2 months
Mumps*	2 months
Mastoidectomy	2 months
Pleurisy—in bed 3 weeks	"in 3d month"

* Patient of Dr. Richard Tefft.

In addition, in this survey they noted the following possibly relevant findings: 3

provocative. In addition, this finding is even more interesting in consideration of associated congenital anomalies other than primary mongolian stigmata (which included 12 instances of deformed little fingers) as follows: imperforate anus, 3 cases; webbing of the fingers, webbing of the toes, and cleft palate, 1 instance of each. The correlation of these deformities with the formation of these particular structures at about the eighth week of fetal life is noteworthy. Ladd and Gross state that "membranous imperforate anus results from a persistence of the anal membrane, an arrest at about the eighth week."³⁰

TABLE 4.—RUBELLA IN RELATION TO 4 CASES OF ABORTION AND STILLBIRTH

Case	Length of pregnancy before rubella	Estimated death of the fetus	Hemorrhage before delivery	Delivery of dead fetus
1	8 weeks	6 months	2 weeks	8 months
2	16 weeks	5½ months	1 week	6 months
3	Uncertain	Uncertain	None	9 months
4	4 weeks	Immediate	None	6 weeks

mothers were respectively noted in hospital records as being "sick throughout entire 9 months," "continually vomiting," and "sick all 9 months, nausea and vomiting, pain in the right side—hemorrhaged before delivery." A fourth had "grippe" during the sixth month, a fifth an "afebrile rash, last trimester," and a sixth was an undoubted alcoholic. A seventh mother had a cholecystectomy between a second pregnancy (normal) and a third (still-born), followed by a fourth pregnancy (mongolian). One mother with 8 normal children had a ninth (miscarriage at 3 months), followed by a tenth pregnancy (mongolian). In this case there was "pink staining at the time of period, second month." Of 50 mongols then, there were 5 instances when a specific, relatively incisive maternal illness was present at the eighth week of gestation, and 5 others in which a more chronic maternal disorder included the eighth week of fetal life.

The number of cases in which there was intercurrent disease, may not be significant in itself, but the tendency to localization of the particular maternal disease at a specific stage of pregnancy is

According to Gray "the limbs begin to make their appearance in the fourth week as small elevations or buds at the side of the trunk. The axial part of the mesoderm of the limb-bend becomes condensed and converted into its cartilagenous skeleton. By the sixth week the three chief divisions of the limbs are marked off by furrows—the upper into arm, forearm, and hand; the lower into thigh, leg, and foot." In the phalanges ossification begins in the body about the eighth week. And again, "the deformity known as cleft palate results from a non-union of the palatal process." "The union . . . commences in front, the premaxillary and palatal processes joining in the eighth week, while the region of the future hard palate is completed by the ninth."

Ingalls²⁶ is making a further analysis of mongolism from the point of view of its embryologic development.

Rubella—General Considerations. Rubella now takes on the status of a disease worthy of the best in diagnosis, reporting, treatment, prevention and research. With this in mind, a reconsideration of the disease is presented.

CLINICAL MANIFESTATIONS. Rubella is a mild, infectious, self-limited disease, ostensibly of the upper respiratory tract, though as is now obvious, of a constitutional nature. Its clinical importance lies in its rare complications. These include secondary infections, encephalitis, neuritis, and now the fetal disturbances already mentioned, and even death.

The infectious agent is a filterable virus, and filtered nasopharyngeal washings give rise to the infection in susceptible human beings upon subcutaneous injection.²² The virus particles are demonstrable by fluorescent microscopy in nasal and pharyngeal washings, in blood and in blister fluid of infected patients.²³ Habel²⁰ has inoculated macacus rhesus monkeys subcutaneously, intraperitoneally, intranasally, and intravenously, with the subsequent development of a mild infection characterized by a leukopenia, relative lymphocytosis and fever (average $5^{\circ}\text{C}.$), followed by a "very light scattered macular rash on the face, abdomen and thighs, starting about the eleventh day, and followed by desquamation." He was also successful in culturing the virus on the chorio-allantoic membrane of the chick embryo in 2 of 4 attempts.

The natural incubation period for man is generally considered "14 to 21 days; usually about 16 days."¹⁹ In an outbreak of rubella in a boys' boarding school¹² 100 of the 106 cases in the first wave fol-

lowed the initial case by 15 to 23 days (*i. e.*, 18 ± 3 days from rash to rash). Peak incidence occurred at 18 days, and there were just 18 days between the theoretical peak of this primary wave, and the peak incidence of a subsequent secondary wave (Chart 1). Thus the interval between cases when it can be established is sufficiently distinct from that of measles, for example, to be used in differential diagnosis.

The prodromal stage is ordinarily 12 to 24 hours though it may be prolonged beyond this period and is often characterized by the development of mild "grippy" malaise, coryza and conjunctivitis, together with enlargement of the post-auricular nodes. In a series of 305 cases, Humphrey²⁴ found that "the most constant symptom was post-auricular adenopathy."

However, it must be stressed that constitutional symptoms may be slight and in school epidemics it has been observed that many infected boys may not even bother to come to sick call.

Most definitions of rubella define the condition as a febrile disease and Humphrey reported in his series that "the temperature ranged from 99° to $100^{\circ}\text{F}.$ in the majority of cases, and remained elevated only 1 or 2 days. In a few instances it rose to 101° and in still fewer to 102° or even $103^{\circ}.$ " In Chart 2 the temperature curves of 20 adolescent boys

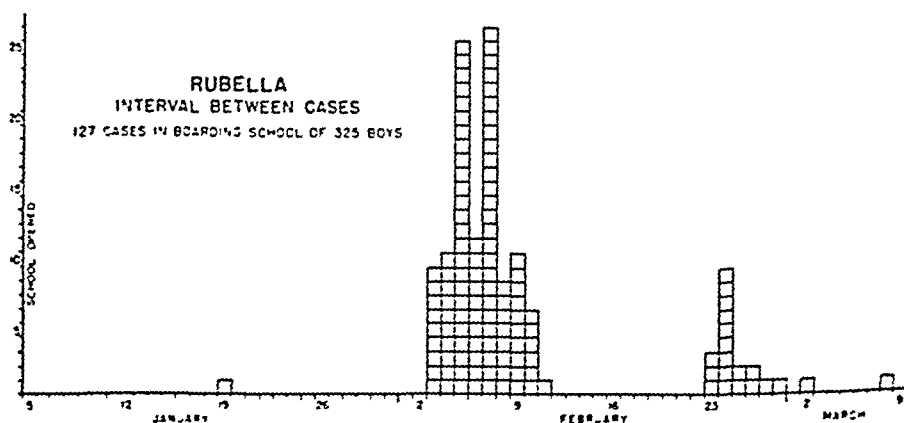


CHART 1.—Boarding school outbreak of rubella, indicating interval between cases averaging 15 days as measured from rash to rash.

selected at random are shown. They are superimposed in groups of 5, and roughly grouped according to whether the temperatures are subnormal, normal, elevated but with a quick return toward normal, and elevated with a slower return toward normal. There appears to be no characteristic curve and many curves are afebrile, or even subnormal throughout the entire course of the disease.

abdomen, and extremities. Occasionally the macules will be as large as a pea, rose-red or darker, and may coalesce.

If the characteristics of the disease cited above appear to give the impression that rubella is difficult to diagnose, it is perhaps well to emphasize at this point that the ordinary case is readily diagnosable and easily differentiated from measles and scarlatina. The exceptional case could be

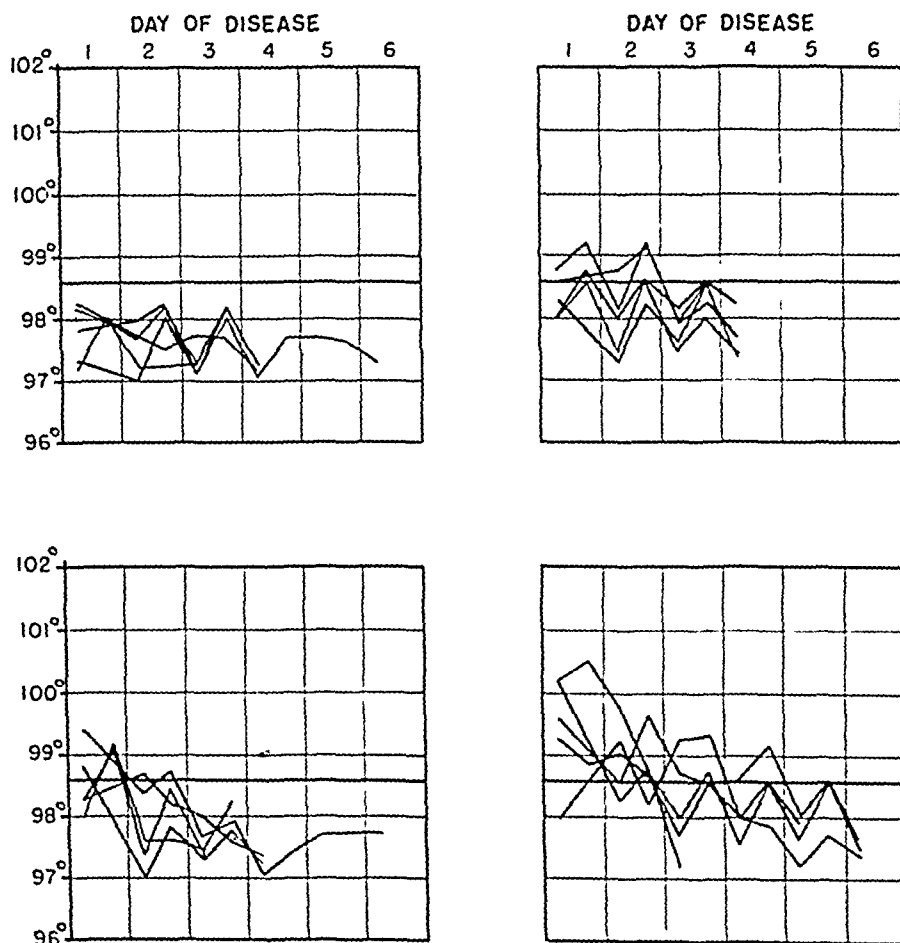


CHART 2.—Temperature curves observed in 20 cases of rubella arranged roughly according to type.

The mucous membranes of the nasopharynx are typically injected, though there is no true enanthem. The bulbar conjunctivæ are suffused so that the white of the eye is more generally pink than inflamed as in measles. The exanthem developing at the end of the prodromal stage is ordinarily manifested as a fine rose-pink maculopapular "pin point" rash, usually beginning on the face and becoming prominent over the trunk,

perhaps more easily confused with a drug rash, particularly if the patient had taken a barbiturate during the prodromal period, and there were no history of exposure.

On the other hand, it must be admitted that none of these signs are pathognomonic or always present. Habel produced infection in monkeys from the nasopharyngeal washings of a 4 year old boy in a physician's family, who was definitely exposed to rubella for 3 consecutive days. 17 days

prior to the development of "fever, malaise, and slight rhinitis followed by posterior cervical lymphadenopathy," yet the child never exhibited a rash. Floystrup¹⁶ also reported the "inapparent" infection (*i. e.*, without rash) of his own son, 2 weeks following exposure to rubella. Two weeks later the boy's brother developed the typical picture. The value of accurate knowledge of the incubation period, and the dates of exposure in such circumstances is apparent.

Hynes²⁵ made leukocyte counts on 37 males, 15 to 39 years old, and on 24 females, 18 to 45, during the first 2 weeks of the disease. He found that a leukopenia was the rule at the onset, with the leukocyte count rising to the upper limit of normal by the tenth day. At the onset, half the patients had an absolute neutropenia, which gradually became corrected. There was also an absolute lymphopenia at the onset, but an absolute lymphocytosis after the fifth day. Türk cells were always present and commonly numerous, and reached their maximum about the fourth day. Plasma cells were present in a half to two-thirds of the patients during the first week. The sedimentation rate was usually normal.

British authors lay much stress on the finding of Türk cells in the blood smear, though it is Diamond's opinion¹¹ that such findings being non-specific would be of no decisive aid in diagnosis. It is apparent that no adjunct to correct diagnosis should be omitted when rubella occurs during pregnancy. Careful search for possible exposure 18±3 days before the rash, careful examination, prompt consultation before the rash disappears when the history or signs are equivocal, are the obviously indicated procedures. Advances in virology, and serological tests are much needed for aid in diagnosis in such cases.

COMPLICATIONS. Secondary Infection. It might be expected that a constitutional virus infection, involving the mucous membranes of the upper respiratory tract, would have secondary infections such as mastoiditis, sinusitis, and the like. Such

does not seem to be the fact. Wesselhoeft after long experience with contagious disease believes rubella to differ strikingly from measles in this respect.⁴⁴ The National Research Council³⁴ mentions no such complications among its 1500 reported cases.

Secondary infections are, however, occasionally to be reckoned with. Thus in Humphrey's experience²⁴ with 305 cases occurring among institutionalized children, 5% developed tonsillitis, another 5% developed non-suppurative adenopathy, 1% suppurative adenopathy, and 1% otitis media.

One of us has seen 5 patients in the post-eruptive stage of rubella contract the typical angina and exanthem of scarlet fever. This occurred on a troopship when a patient immune to rubella, suffering from a streptococcal ulcer of the leg, was moved into the sick bay with rubella patients because of lack of space elsewhere. In view of the relative rarity of secondary infection, their variety and the circumstances under which some of them occurred it is a question whether these instances represent actual "complications" or merely coincident infections.

Neuritis. There are rare reports in the literature^{21,23} of what has been diagnosed clinically as "neuritis" appearing 3 to 6 days after the appearance of the rash. Pain, numbness or paresthesias of the wrists, shoulders, fingers, hands or ankles, has been complained of by the patient, and in about half the cases transient motor weakness was detected. In 4 such patients where the age is given the average was 32. There is some suggestion that the phenomenon was "arthritic" because of joint involvement and swelling, rather than a true neuritis. The cases were seen in general practice and no one has reported the condition in school epidemics. We have encountered one such case with "rheumatic" puffiness of wrists and ankles for a week following rubella.

Encephalitis. Margolis, Wilson and Top²¹ estimated that encephalitis occurs in approximately 1 out of 6000 cases of

rubella. Patients with this complication exhibit the non-specific signs of encephalitis: headache, vomiting, stiff neck, fever, reflex or speech changes, and the like. The authors note that "the leukopenia of rubella becomes a leukocytosis with the onset of encephalitis." Severe and persistent convulsions characterized the course of their 4 fatal cases, and were encountered in 4 of their 10 patients who recovered. The course, whether mild or severe, was found to be short, and death when it occurred (20.8% fatality rate) was within 3 days of the onset of encephalitic signs. These occurred "with striking consistency 4 days after the onset of the disease." They further note that only 1 of the 34 previously reported cases in the literature developed persisting sequelæ—ataxia, of all the extremities, tremor of the right hand and partial facial paralysis. These symptoms cleared in 2 months. One of the authors' cases had similar persisting signs 4 months after onset.

There does not appear to be any special predilection of encephalitis for any one age group. Encephalitis is not mentioned as having been encountered in the more than 1500 cases studied by the British Medical Research Council in boarding schools.

RECRUDESCENCES. The great majority of recurrent attacks follow shortly in the wake of the first. Thus in an epidemic reported by Humphrey²⁴ 19 patients (6%) had recrudescent attacks which occurred from 12 to 41 days after the onset of the first attack. He states "that in our opinion there can be no question concerning the diagnosis. In such instances . . . the child went through the original course of the disease without complication, and then later a second course with similar symptoms, except that in 3 complications developed. . . . The rash was typical and generalized in both of the attacks. It was observed that in general the second attack was of shorter duration and was milder. It should be noted that there was opportunity in the institution for re-exposure."

He concluded that "the large number of second attacks suggests that immunity in rubella may not develop for about 3 weeks after onset." The average interval excluding the 1 case with a 41-day intervening period, was 26 days.

In one outbreak of 65 cases brought to our attention, one recrudescent attack occurred following the primary by an interval of 18 days.

SECOND ATTACKS. Bona fide second attacks of rubella appear to be rare. That they do occur is suggested by the National Research Council's statement that "in 4 pairs of epidemics analyzed in detail, 2 boys at school N.B. were attacked in both epidemics, the diagnosis being made by the same school medical officer." However, physicians, nurses and parents are continuously being re-exposed without contracting the disease for a second time and the very nature of the epidemiologic curves which are presented here show that immunity is essentially lifelong. It is probable that the recrudescent cases described above account for the widespread impression that rubella is readily contracted for a second time. Misdiagnosed cases and misinformation add to the confusion on this point. Thus the National Research Council finds that "in 9 analyzed epidemics among boys and in 3 among girls, the attack rate on so-called immunes was over 10%." However, the Council doubted that "error can account entirely for this phenomenon."

INAPPARENT ATTACKS. There is some evidence that a susceptible individual may acquire immunity to rubella without apparent clinical signs. The National Research Council notes that "in one school, out of 539 boys not attacked in 1932 when the attack rate was 17.1%, 339 were still in school in 1933 when there was a second outbreak. None of these boys was attacked in the second outbreak, whereas 20.4% of the boys exposed for the first time contracted rubella. Perhaps the true explanation lies in the ease with which mild clinical signs may be overlooked.

Rubella—Epidemiology.* In view of the long-held attitude toward German measles as a "nuisance rather than a disease," it is not surprising that little information is to be found which affords much of a conception of the epidemiologic behavior of the disease. Beyond the fact that it is obviously of a contagious nature, data have not been assembled which establish even such general features as the extent of occurrence of the disease, its

forms an epidemiologic pattern sufficient to stamp it as one of "the immunizing infections of childhood" of the same magnitude as measles. In Chart 3 is shown the reported incidence of the disease in Massachusetts from 1917 to 1945 by years and by months. There is a fairly regular periodicity differing from measles in that high prevalence tends to extend over periods of 2 or 3 years in sharp contrast with the intervening low prevalence of

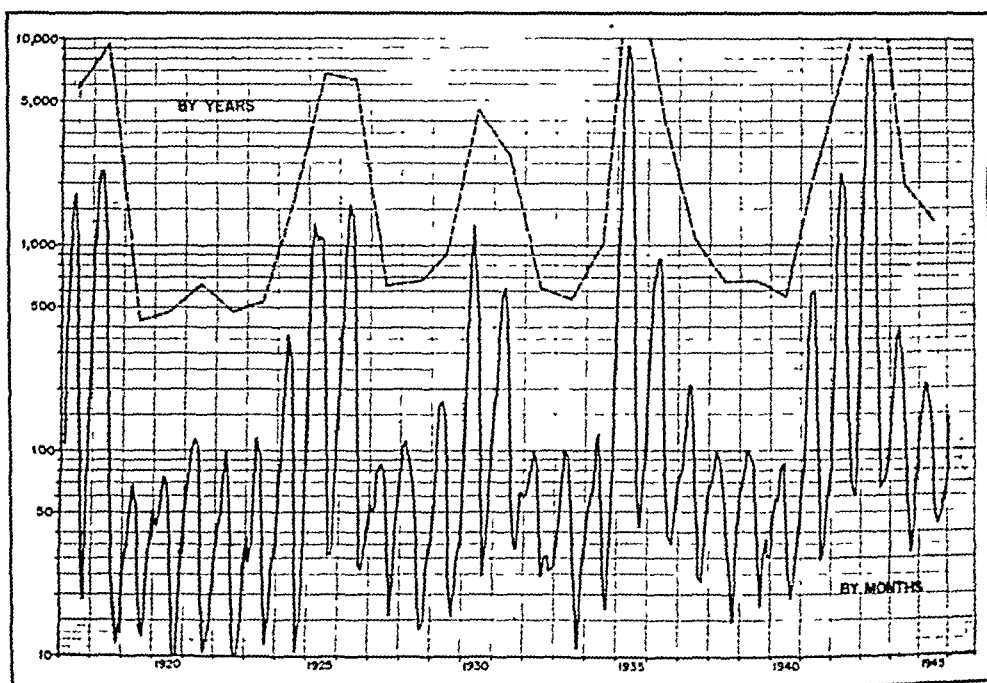


CHART 3.—Reported incidence of rubella by years and by months in Massachusetts.

age distribution, urban-rural behavior, duration of immunity, etc. But on reading the foregoing discussion on incubation period, recrudescences, second attacks and inapparent attacks, one is struck by the similarities to measles. From data presented here, it would be expected that equally complete reporting and study would reveal an epidemiologic pattern in German measles not unlike that of measles.

There are many indications that the reporting of German measles is for apparent reasons highly deficient. Nonetheless the reported occurrence of the disease

2, 3 or 4 years' duration. This is seen in the seasonal curve, similar to that of measles, excepting that during the periods of high prevalence the disease remains at distinctly high levels in the "off season." Not only these general features but the fact that in the highest years the reported cases of German measles attain the same proportion as the highest years of measles is especially suggestive of an extent of spread in German measles similar to that in measles. In lower years the fact that cases run only about one-tenth those of measles is believed to be due to deficiency in reporting.

* We are indebted to the Division of Communicable Diseases, State Department of Health of Massachusetts for epidemiologic data on rubella, and to Miss Katharine H. Hendrie for analysis of it.

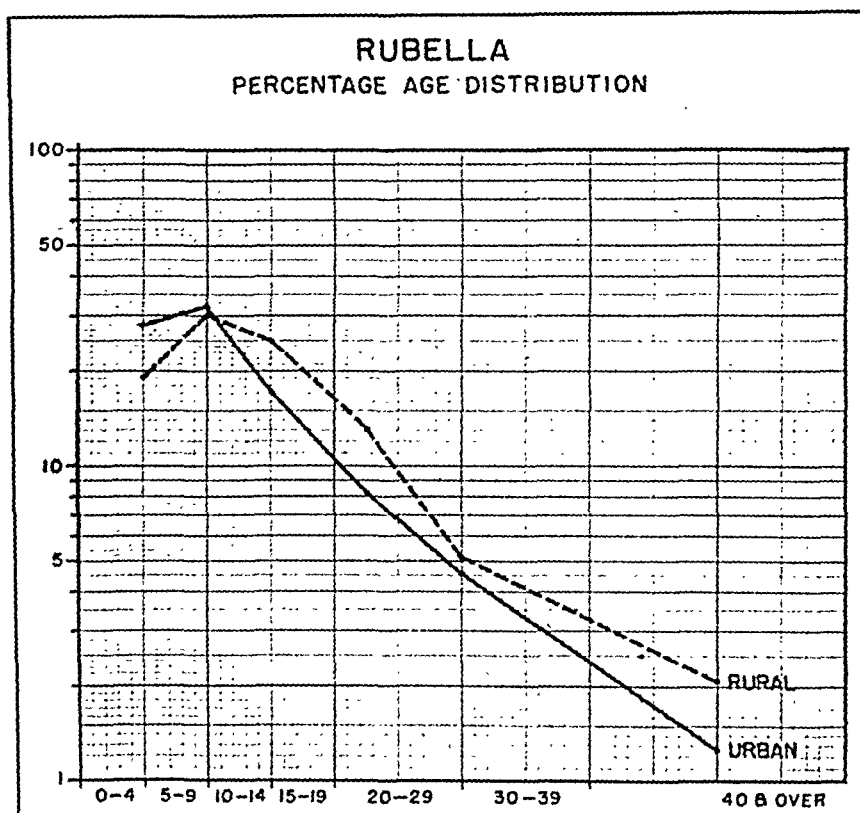


CHART 4.—Comparative age distribution of rubella in urban and rural Massachusetts in 1943.

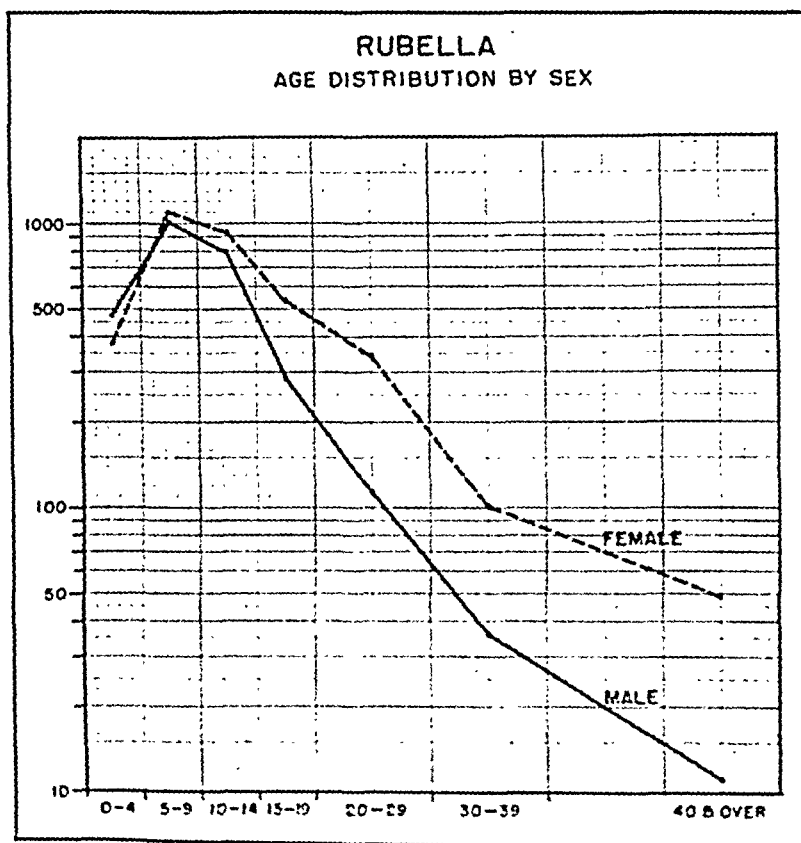


CHART 5.—Reported cases of rubella by age groups and sex in Massachusetts, 1913.

AGE AND SEX DISTRIBUTION. The percentage age distribution of rubella in Massachusetts in 1943 for seven largest cities and for towns under 5000 are shown in Chart 4. The occurrence of the disease at a somewhat older age in smaller populations is characteristic of the "immunizing infections of childhood."

In both the urban and rural population the reported cases in females become increasingly greater in the older age groups (Chart 5). To what extent the excess in females in the older age groups may be due to better reporting by reason of the fact that females are in the home and are reported along with smaller children or to what extent there is greater exposure to children as suggested by Pope's³⁵ study of secondary attack rates in scarlet fever is not known. In the present connection, the point of interest is the fact that about 14% of female cases and about 8% of all cases are in females of child-bearing age.

Finally it might be stated that there is no seasonal differential between German measles and measles. The curves of seasonal prevalence are closely similar.

Prevention of Anomalies. At the present time the only positive measure is deliberate exposure before the child-bearing period. It would seem undesirable to prevent the naturally occurring infection among young people when their mothers, if susceptible, were not pregnant; and in females actually to seek exposure if they have not contracted the disease by adolescence. It is possible that inoculation* with rubella may be part of the pediatrician's future program of preventive medicine,† just as is vaccination against smallpox. Such a possibility awaits the development of suitable techniques. There is a mortality to be reckoned with:‡ something in the order of 1/30,000 to 1/100,000, but this mortality is to be found in all age groups and the menace that the disease holds

* If it took 100 years to uncover the severe sequelæ of rubella, oddly enough we may have to go back 100 years to uncover a principle applicable to their prevention: namely, inoculation. Variolation, "that most interesting epoch in medical history which kept greater and humbler minds in fever heat during almost one whole century," has entered little, if at all, into later immunologic investigation,³⁶ although it called forth, in its time, a unique and extensive trial of a specific preventive measure, the logical consequence of which was vaccination. Without attempting to review the activities of many great medical minds of the day, it is interesting that they were extended to such figures as Lady Mary Wortley Montague, Benjamin Franklin, Cotton Mather, Voltaire and Frederick the Great, not to mention the brothers Robert and Daniel Sutton, themselves not medical men, who became specially famous for their "successful inoculation." With many attempts to make it safe by preparation of the patient, such as the cooling regimen of Sydenham so that the "solids and fluids may be reduced from a greater to less inflammability," or modifying the inoculation by dilution and the use of "passage" or "unripe" virus, it might well have furnished an efficacious preventive measure,³⁷ had it not been quickly supplanted by the safe and practicable vaccination. It was declared a felony in England by an Act of Parliament in 1810.³⁸ In the manner that the name vaccination came to designate an immunological principle, "variolation" as a principle may be revived (inoculation with the virus of rubella) as constituting the most feasible procedure available at the present time for the control of the disastrous sequelæ of rubella.

Variolation at best was a drastic procedure, and would have been so even if strains like *alastrim* had been utilized. Its objective was to induce life long immunity by mild strains (based on crude empirical criteria) at the most propitious time and under the most propitious circumstances. Measles is another inevitable disease in which deliberate exposure has been practiced at times, particularly within the family circle. The use of immune globulins permits the physician to postpone the inevitable if the moment is not propitious, or to provoke a milder disease. The possibility of inoculation of infants before passive immunity of maternal origin disappears or the production of modified measles by the combined use of globulin injection and virus inoculation, has not been explored, no doubt for the reason that virus has not been available. A fraction of the population gives no history of ever having contracted rubella, even after known exposure. It is conceivable that such people may have contracted an unrecognized modified form of the disease while they yet possessed partial maternal immunity.

† Incidentally, the practice of inoculation with, or exposure to rubella would provide an unprecedented opportunity for observation on the precise mechanism of natural infection: that aspect of epidemiology which has been least satisfactorily imitated in experimental disease in animals. For example, inoculation or exposure of a group of volunteers is the only satisfactory way of determining the efficacy of gamma globulin as a prophylactic, short of extensive and laborious studies such as have been made on measles during the past decade. A defect inherent in this latter type of investigation is that the differential diagnosis between modified measles and rubella is so tenuous that the differentiation of a failure to immunize from modified measles could occasion considerable confusion.

‡ Figures tending to show that the mortality is 1 to 60,000 may be excessively pessimistic, as it is probable that most cases of post-rubella encephalitis are reported whereas a minority of cases of the uncomplicated disease are thought to be reported.

for the unborn child would be appreciably reduced if not eliminated.

The only prophylactic therapy that can be proffered an exposed pregnant mother is immune globulin. However, available clinical experience has not established its efficacy. There have been a number of instances of evident failure of gamma globulin to prevent the disease. The term "evident failure" is used, since the distinction between modified measles and rubella is not an easy one: rash, Koplik spots, and incubation periods all being altered. In one family, 2 children exposed to both measles and rubella in school developed a fine rash and post-auricular adenopathy 14 and 15 days after an injection (2.5 cc.) of gamma globulin. Of 2 other children in the same family, 1 was given gamma globulin at the time the first cases developed; the other a 6 months old baby was not. Both developed typical rubella 15 days after the first sibling's rash. The mother who had had measles

in the past but not rubella also developed typical rubella.⁴ There appears to be little reason for believing the disease in the three "failures" was modified measles.

Many authors have gone as far as to suggest that "the justification for therapeutic abortion," if rubella (or other exanthemata) be contracted in the first 2 months of pregnancy, "should be debated." Others have gone so far as to accept the available evidence as sufficient grounds for termination of the pregnancy.

Such a far-reaching question can be approached with wisdom only when there are adequate statistical studies to establish the specific risks of infection at all stages of pregnancy. Knowledge from such studies would have to be interpreted not only in terms of actual risk of congenital anomalies but as well in terms of the "health of the mother" in continuing a pregnancy with such a known risk and finally in terms of an informed public opinion.

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BOOK REVIEWS AND NOTICES

NURSING IN COMMERCE AND INDUSTRY. By BETHEL J. McGRATH, R.N. Pp. 355. New York: Commonwealth Fund, 1946. Price, \$3.00.

WITHOUT defining objectives, the author goes straight to the point indicated in the title and presents a manual, complete, well organized and delightfully written. As such, it fulfills a long felt need of nurses in industry. However, the book far transcends these limits. It holds much of interest to all nurses, as well as to executives in industry, to the field of preventive medicine and public health, and to the general public, and also meets the need for a guide to those concerned with the development of health services for students, staff and other personnel in hospitals, schools of nursing and medicine. We note especially the democratic and thought-provoking chapter on relationships with employees and employer organizations. The question of union membership, presented with fairness and restraint, still retains its question mark; the Reviewer would have appreciated stronger support of the professional attitude.

H. F.

AN INTRODUCTION TO ESSENTIAL HYPERTENSION. By RICHARD F. HERNDON, M.D., F.A.C.P. Pp. 88; 7 illus. Springfield, Ill.: Thomas, 1946. Price, \$2.50.

THIS little book is clearly written, with frequent summaries and excellent illustrations. It condenses a great deal of material in a relatively small space, and hence should be of value to the physician who wishes to acquire a viewpoint quickly, the physician whom the author describes in the Preface as "the ordinary practitioner on the firing line of medicine." It contains no new or original material, and will probably be of little use to the physician already interested in hypertension. The limitation of space that the author has set for himself necessitates certain omissions that may give the casual reader a distorted view of the outlook for the future.

J. G.

THE VENOUS PULSE AND ITS GRAPHIC RECORDING. By FRANZ M. GROEDEL, M.D., Attending Cardiologist, Beth David Hospital. Pp. 223; 7 ills. and 290 tracings. New York: Brooklyn Medical Press, 1946. Price, \$5.50.

THE techniques and interpretations of more than a century of investigation of phlebography, pneumocardiography and esophagocardiography are described and criticized. Technical improvements of methods are demonstrated by many excellent tracing reproductions. An extensive bibliography is provided. This book is difficult reading. Its evaluations are in some cases open to question (cf. ballistocardiography). The author is diffuse and repetitive, and seems to overstress the value of these methods in clinical investigation. C. K.

COSMETICS AND DERMATITIS. By I. SCHWARTZ, M.D., and S. M. PECK, M.D., U. S. Public Health Service. Pp. 189; 20 ills. New York: P. B. Hober, 1946. Price, \$4.00.

THE authors concisely present a satisfactory analytical approach to skin reactions to popular cosmetics. The book contains several uninformative photographic illustrations and statements, especially in Chapter I, such as (p. 18): "The hairs which grow in the nostril have the general character of eyelashes. They are only infrequently found in women;" and (p. 19): "Most people have hair on the extremities. In childhood transitional hairs are found. They are later replaced by terminal hairs like those on the body, but shorter," which tend to decrease rather than increase the reader's interest. Nevertheless, this interest is so amply rewarded in subsequent chapters, that the book is a really valuable contribution to medical literature and should be read and re-read by every physician practicing dermatology, as a specialist, or general practitioner.

J. L.

ESSENTIALS OF GENERAL ANESTHESIA. By R. R. MACINTOSH and FRED A. BANISTER, University of Oxford. Third ed. Pp. 334; 239 ills. Springfield, Ill.: Charles C Thomas, 1945. Price not given.

In this third edition, relatively few changes have been made in the text. The book is primarily intended for dentists, but all of the material presented is of interest to the anesthesiologist regardless of the type of surgery contemplated. R. D.

CURRENT THERAPIES OF PERSONALITY DISORDERS. Proceedings of the Thirty-fourth Annual Meeting of the American Psychopathological Association, held in 1945. By BERNARD GLUECK, M.D., Editor. Pp. 300. New York: Grune & Stratton, 1946. Price, \$3.50.

THE Presidential Address was entitled: Psychiatry, an Instrument of Personal and Social Rehabilitation. Following this were papers by the various collaborators under these captions: I, The Modern Psychiatric Hospital; II, The Psychochemical Techniques in Psychiatry; III, The Psychiatric Guidance Rehabilitation Techniques.

Group psychotherapy is slowly making headway, so that children, adolescents and adults may be benefited by its application. Relationships with others than their own families are important to children, and group play supplies such a need. Adolescent delinquents may receive benefit. Among adults, the neuropsychoses and mild types of functional psychoses are suitable for group therapy. But not all subjects should receive such treatment; where there are deep-rooted emotional conflicts, perhaps the psychoanalytic method may prove more helpful. Alcoholics Anonymous with its personal, religious and social implications, receives favorable consideration. The employment of convulsive shock therapy in some psychotics, also to prevent deterioration and to maintain certain behavior levels, may prove gratifying when proper amounts are adequately administered, and with careful management of the patients.

Though these contributions are excellent, no book can be all-inclusive. We would have liked to have seen Moreno's psychodramatics considered. N. Y.

NEW BOOKS

Environmental Warmth and Its Measurement. By T. BEDFORD, D.Sc., Ph.D., M.I.-MIN.E. Pp. 40, Supplement of Charts for the Calculation of Environmental Warmth (H.M.S.O.). London: British Information Services, 1946. Price, \$70.

Women in Industry, Their Health and Efficiency. Issued under the auspices of the Division of Medical Sciences and the Division of Engineering and Industrial Research of the National Research Council. Prepared in the Army Industrial Hygiene Laboratory by ANNA M. BAETJER, Sc.D., Assistant Professor of Physiological Hygiene, School of Hygiene and Public Health, The Johns Hopkins Hospital. Pp. 344. Philadelphia: Saunders, 1946. Price, \$4.00.

Curare Intocostrin. Composed of reports of one hundred and forty-eight investigators and clinicians published in recent years. Pp. 292. New York: E. R. Squibb & Sons, 1946. No Price Given.

Advancing Fronts in Chemistry. By WENDELL H. POWERS (edited), Assistant Professor of Chemistry. Pp. 156. New York: Reinhold Publishing Corporation, 1946. Price, \$3.25.

Medical Service by Government. Local, State, and Federal. By BERNHARD J. STERN, Ph.D., Lecturer in Sociology, Columbia University. Pp. 208. New York: The Commonwealth Fund, 1946. Price, \$1.50.

New Aspects of John and William Hunter. By JANE M. OPPENHEIMER, Bryn Mawr College. With a Foreword by FENWICK BEEKMAN, M.D. Pp. 208; 5 ills. New York: Henry Schuman, 1946. Price, \$6.00.

Wm. Beaumont's Formative Years. With Annotations and an Introductory Essay by GENEVIEVE MILLER, M.A., Institute of the History of Medicine, The Johns Hopkins University. Pp. 87. New York: Henry Schuman, 1946. Price, \$6.00.

Squint and Convergence. By N. A. STUTTERHEIM, M.D. (Rand), State Medical Qualification, Holland; formerly Surgeon to the Eye Clinic, University, Leyden; Part-time Ophthalmic Surgeon to the Johannesburg School Clinic, Education Dept., Transvaal. Pp. 95; 26 graphs; 15 diagrams. London: H. K. Lewis & Co., Ltd., 1946. Price, 15/-net (\$3.00).

Skin Diseases, Nutrition and Metabolism. By ERICH URBACH, M.D., F.A.C.A., Associate in Dermatology, University of Pennsylvania School of Medicine; Chief of Department of Allergy, Jewish Hospital, Philadelphia. With the collaboration of EDWARD B. LEWINN, B.S., M.D., F.A.C.P., Associate in Medicine, Jewish Hospital, Philadelphia. Pp. 634; 266 ills. New York: Grune & Stratton, Inc., 1946. Price, \$10.00.

Diagnostic Examination of the Eye. By CONRAD BERENS, M.D., F.A.C.S., Professor of Clinical Ophthalmology, Columbia University; Executive Eye Surgeon, New York Eye and Ear Infirmary; formerly Chairman, Section on Ophthalmology of the American Medical Association; Managing Director, The Ophthalmological Foundation, Inc.; President, Snyder Ophthalmic Foundation; formerly Chairman, American Board of Ophthalmology; Consultant to the Air Surgeon of the Army Air Forces; and JOSHUA ZUCKERMAN, B.Sc., M.D., C.M.I., F.A.C.S., Instructor in Ophthalmology, New York University; Instructor in Ophthalmology, New York Eye and Ear Infirmary and Columbia University Postgraduate School; Ophthalmic Surgeon, Midtown Hospital; Assistant Ophthalmic Surgeon, New York Eye and Ear Infirmary; Diplomate, American Board of Ophthalmology; Fellow, American Academy of Ophthalmology and Otolaryngology. Pp. 711; 410 ills. Philadelphia: J. B. Lippincott Company, 1946. Price, \$15.00.

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A History of Medicine. By DOUGLAS GUTHRIE, M.D., F.R.C.S.Ed., F.R.S.E. With an Introduction by SAMUEL C. HARVEY, M.D., F.A.C.S., Wm. H. Carmalt Professor of Surgery, Yale University School of Medicine. Pp. 449; 72 ills. Philadelphia: J. B. Lippincott Company, 1946. Price, \$6.00.

- Psychotherapy in General Medicine.* By GEDDES SMITH, Associate, The Commonwealth Fund. Pp. 38. New York: The Commonwealth Fund, 1946. Price, \$8.25.
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- The Diagnosis and Treatment of Pulmonary Tuberculosis.* By MOSES J. STONE, M.D., Assistant Professor of Medicine, Boston University School of Medicine; Instructor in Medicine, Tufts Medical School; Physician-in-Chief, Chest Clinics, Beth Israel Hospital and Massachusetts Memorial Hospitals, Boston; and PAUL DUFAULT, M.D., F.A.C.P., Superintendent of the Rutland State Sanatorium, Rutland, Mass. Pp. 325; 93 engr. Philadelphia: Lea & Febiger, 1946. Price, \$3.50.
- Pneumoperitoneum Treatment.* By ANDREW LADISLAUS BANYAI, M.D., F.A.C.P., F.C.C.P., Associate Clinical Professor of Medicine, Marquette University Medical School, Milwaukee, Wis.; Member, Editorial Board, "Diseases of the Chest;" formerly Preceptor in Tuberculosis, School of Medicine, University of Wisconsin, Madison, Wis. Pp. 375; 78 ills. St. Louis: The C. V. Mosby Company, 1946. Price, \$6.50.
- Ocular Muscles and Fusion.* By THOMAS G. ATKINSON, M.D. Pp. 192. Chicago: The Professional Press, Inc., 1946. Price, \$3.50.
- Studies in Hypertony and the Prevention of Disease.* By I. HARRIS, M.D., Honorary Director, Institute for Prevention of Disease; Honorary Physician, Liverpool Heart Hospital. In Coöperation with J. T. IRELAND, B.Sc. (Hons.), A.I.C., Leverhulme Research Fellow; G. V. JAMES, M.Sc., A.I.C., Maurice Stern Research Fellow; EDWARD CRONIN LOWE, M.B.E., M.B., B.S., Director, Pathological Dept., Southport Infirmary; Honorary Clinical Pathologist, Institute for Prevention of Disease; C. E. VERNON, M.Sc., A.I.C., Research Fellow. Pp. 114. Baltimore: The Williams & Wilkins Company, 1946. Price, \$3.00.
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- Proceedings of the Conference on Diagnosis in Sterility.* Edited by EARL T. ENGLE, Chairman, Committee on Research, National Committee of Maternal Health. Pp. 237. Springfield: Charles C. Thomas, 1946. Price, \$5.00.
- The American Hospital.* By E. G. L. CORWIN, Ph.D., Executive Secretary, Committee on Public Health Relations, The New York Academy of Medicine; Honorary Charter Fellow of the American College of Hospital Administrators; Former Secretary General and Honorary President of The International Hospital Association. Pp. 226. New York: The Commonwealth Fund, 1946. Price, \$1.50.
- Peripheral Vascular Diseases.* By EDGAR V. ALLEN, B.S., M.A., M.D., M.S. in Medicine, F.A.C.P., Division of Medicine, Mayo Clinic; Associate Professor of Medicine, Mayo Foundation, Graduate School, University of Minnesota; Diplomate of the American Board of Internal Medicine; NELSON W. BARKER, B.A., M.D., M.S. in Medicine, F.A.C.P. Division of Medicine, Mayo Clinic, Associate Professor of Medicine, Mayo Foundation, Graduate School, University of Minnesota; Diplomate of the American Board of Internal Medicine; EDGAR A. HINES, JR., M.D., B.S., M.A., M.S. in Medicine, F.A.C.P. Division of Medicine, Mayo Foundation Graduate School, University of Minnesota with Associates in the Mayo Clinic and Mayo Foundation. Pp. 871; 386 ills. Philadelphia: W. B. Saunders Company, 1946. Price, \$10.00.

NEW EDITIONS

- Electrocardiography in Practice.* By ASHTON GRAYBIEL, M.D., Captain, Medical Corps, U. S. Naval Reserve Coördinator of Research, U. S. Naval School of Aviation Medicine, Pensacola, Fla.; and PAUL D. WHITE, M.D., Lecturer in Medicine, Harvard Medical School; Physician, Massachusetts General Hospital. With the Assistance of LOUISE WHEELER, A.M., Executive Secretary, The Cardiac Laboratory, Massachusetts General Hospital; CONGER WILLIAMS, M.D., Assistant in Medicine, Harvard Medical School and Massachusetts General Hospital. 2nd ed. Pp. 458; 323 ills. Philadelphia: W. B. Saunders Company, 1946. Price, \$7.00.

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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

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ORIGINAL ARTICLES

THE THYROID AND BLOOD REGENERATION IN THE RAT

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It is generally known that the thyroid gland exerts some influence over the process of hemopoiesis. Reports on the nature of this relation, however, show many contradictions. For example, thyroidectomy is stated to: (1) cause an anemia;^{2,3,8,11,15,16,19} (2) produce no change in red cell count;^{13,35} (3) cause leukocytosis and relative lymphopenia;^{3,13,16} (4) induce some leukopenia and lymphocytosis.^{4,6,11} Further pertinent information is offered by Mannsfeld,²⁵ and Askanazy,¹ who found that thyroidectomized animals show a slow hemopoietic response to high altitudes, and by Furuya,⁶ who noted a similar delay following hemorrhage. Meyer, Thewlis and Rusch²⁶ have reported, however, that thyroidectomized rats respond to the erythropoietic stimulation of reduced pressures in much the same way as do normal rats. In clinical hypothyroidism most investigators agree that there is an anemia^{2,21,23,24} and a lymphocytosis,^{14,21} but Wilson³⁵ reports a relative lymphopenia. In addition, Tatum²⁷ has found the marrow of cretins to be fatty and Watanabe²⁴ has reported that hypo-

thyroidism results in a disorder of blood formation. Recently, Paul, Limarzi and Seed²⁷ have shown that thyroidectomy depresses erythropoiesis in polycythemic patients.

Studies of the blood picture in experimental and clinical hyperthyroidism also have yielded contradictory results. This condition has been reported to induce: (1) an increase in the number of red cells;^{12,19,22,36} (2) an anemia, often preceded by a rise in erythrocyte number;^{2,6,20,29,36} (3) leukopenia and lymphocytosis;^{4,5} (4) leukocytosis involving granulocytosis;²² (5) lymphopenia, granulocytosis and no change in total white cell count.²

These conflicting reports appear to indicate a need for additional and more highly controlled work on the subject. This investigation is concerned with the effects of hypothyroidism induced surgically or through thiouracil feeding, and of thyroxin on recovery from the anemia produced by hemorrhage in adult male rats. These results will be compared with those obtained with testosterone propionate and

cobalt nitrate. Use has been made before of the bled rat as a sensitive test animal for observing hemopoietic activities of hormonal or vitamin factors.^{7,18} The present experiments include a study of red and white cells, hemoglobin, reticulocytes and bone marrow. A preliminary report on several phases of this work has already appeared.⁹

Materials and Methods. Thyroidectomies were performed under nembutal, and castrations under ether anesthesia. Initial counts of red blood cells (RBC), white blood cells (WBC), hemoglobin and reticulocytes were made on all animals. An anemia was induced by bleeding. From 4.25 to 6 cc. of blood, depending upon the weight of the animal, were withdrawn by cardiac puncture. Complete counts on each animal were made the day following bleeding and at weekly intervals thereafter. Subcutaneous injection of hormones and cobalt nitrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) were begun the day after bleeding and continued daily throughout the course of the experiment.

Peripheral blood from the tail was employed and all counts were made in duplicate. Red cell counts were required to agree within $\pm 4\%$ and white cell counts within $\pm 10\%$. Reticulocyte counts were made from wet mounts of whole blood in 2% sodium citrate solution and stained with brilliant cresyl blue; 500 red cells were counted and the reticulocytes expressed as a percentage of these. Differential counts were made on smears of whole blood treated with Wright's stain. Hemoglobin content was determined with a Fisher electrohemometer.

For the bone marrow studies, the entire right femur was fixed in Downey's solution, decalcified in a mixture of equal parts of formic acid and 20% sodium citrate, dehydrated and cleared in dioxan, sectioned in paraffin and stained with hematoxylin and eosin or Kingsley's polychromatic solution. Smear preparations of marrow from the left femur were also made.

Results. SERIES 1. This consisted of 25 not operated upon and 24 thyroidectomized animals. These were subdivided the day after bleeding into the following

groups: (1) 8 unoperated and 8 thyroidectomized rats receiving no treatment; (2) 8 unoperated and 8 thyroidectomized rats given 0.1 mg. thyroxin; (3) 9 unoperated and 8 thyroidectomized rats given 0.1 mg. thyroxin and 0.5 mg. testosterone propionate.*

1. *Effects of Thyroidectomy.* A survey of the initial counts before bleeding revealed no effect of thyroidectomy on the RBC and WBC 2 weeks after the operation. Hemorrhage caused no greater decrease in RBC than that shown by the unoperated controls. The return of the RBC, however, to normal levels was markedly inhibited in the thyroidectomized untreated group (Fig. 1). Hemoglobin regeneration was also slowed but not to as great a degree. With regard to total white count, the thyroidectomized group was the only one which showed a slow continued increase. However, the level at the end of the experiment was within the limits of the normal range. Reticulocytes appeared to be within normal limits throughout and the differential white cell counts revealed no significant change. The bone marrows of the thyroidectomized animals revealed some hypoplasia accompanied by a fatty infiltration, and a smaller erythroid/myeloid cell ratio than that observed in unoperated rats about 1 month after bleeding (Figs. 4 and 5).

Six rats were subjected to surgical procedure identical with that of thyroidectomy except that the thyroid was not removed. These animals showed a smaller decrease in RBC after bleeding than did the other groups. By the end of the 2nd week pre-bleeding levels were attained in all animals. In most respects this group did not differ significantly from the unoperated controls.

2. *Effects of Thyroxin.* In both the bled unoperated and thyroidectomized animals, thyroxin injections caused an increase in the rate of RBC regeneration (Fig. 1). This increase was relatively greater in the thyroidectomized than in the unoperated

* We wish to thank Dr. Erwin Schwenk, Schering Corporation, for the testosterone propionate.

groups. A marked stimulation of hemoglobin regeneration was noted in the thyroidectomized rats receiving this hormone. No consistent effects on the total or differential white cell count were observed. Reticulocyte numbers were increased above the hemorrhagic levels by thyroxine to a greater degree again in the thyroidectomized than in the unoperated groups. The vacuolar appearance of the marrow observed in thyroidectomized rats was considerably repaired by thyroxine (Fig. 6). The erythroid/myeloid cell ratios as determined from marrow smears were also significantly increased.

0.05 cc. sesame oil; (3) 6 given 0.1 mg. thyroxine; (4) 10 treated with 1.25 mg. testosterone propionate; (5) 6 given 0.1 mg. thyroxine along with 1.25 mg. testosterone propionate; (6) 6 treated with 1.25 mg. cobalt nitrate; and (7) 6 given 0.1 mg. thyroxine and 1.25 mg. cobalt nitrate. The results are shown in Figure 2.

1. *Effects of Thyroxine.* The picture found here was the same as in Series 1, *i. e.*, a more rapid return of RBC and hemoglobin to normal levels, marked reticulocyte response, no change in total and differential WBC count, and a stimulated marrow.

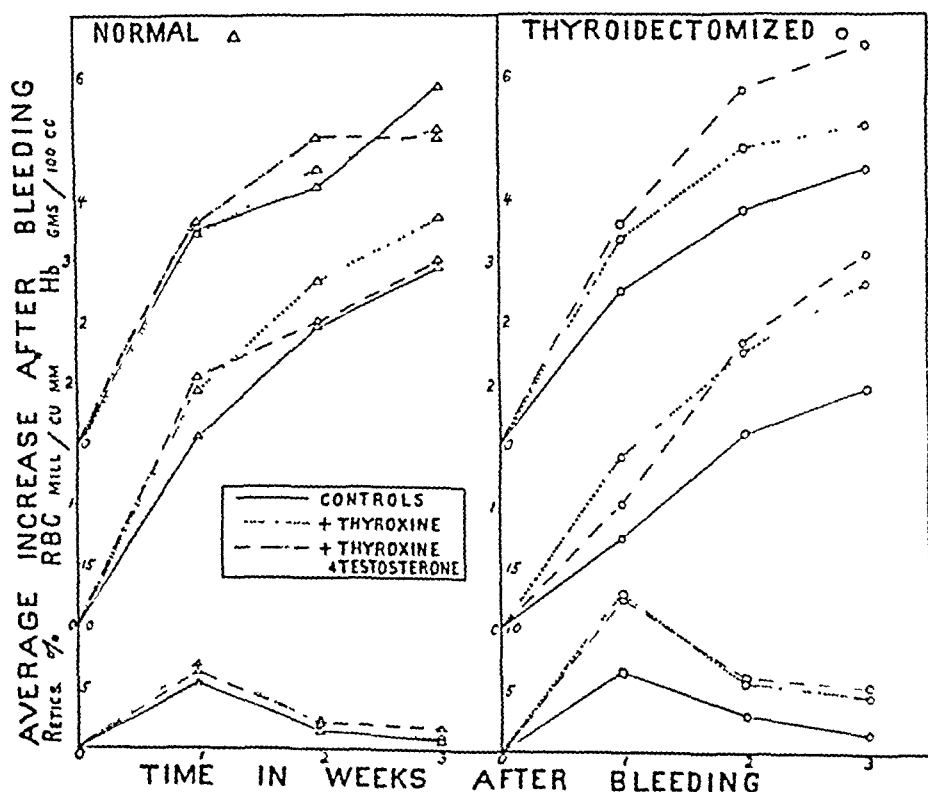


FIG. 1.—Effects of thyroxine and the combination of thyroxine and testosterone on average RBC, hemoglobin values and reticulocyte percentages in normal and thyroidectomized rats following bleeding.

3. *Effects of Thyroxine Plus Testosterone.* This combination of hormones produced the same general picture as thyroxine alone (Fig. 1). It caused, however, a more pronounced rise in hemoglobin in the thyroidectomized group.

SERIES 2. The 44 thyroidectomized animals were subdivided into the following groups: (1) 5 untreated; (2) 5 given

2. *Effects of Testosterone.* This hormone stimulated slightly RBC regeneration but produced a possible inhibition in hemoglobin production. Reticulocyte numbers were higher in this group during the 1st week of treatment. The bone marrow appeared normal.

3. *Effects of Thyroxine Plus Testosterone.* Except for a stronger hemoglobin and

reticulocyte response, the effects of this combination were quite similar to those produced by thyroxine alone.

4. *Effects of Cobalt.* This drug evoked as great a hemoglobin response as did thyroxine, and exerted a more powerful influence on RBC production. Reticulocytes were maintained at higher than normal levels. The bone marrows were markedly hyperplastic in all cases, revealing densely packed areas of erythrogenic cells.

SERIES 3. The animals were subdivided as follows: (1) 7 unoperated untreated; (2) 5 thyroidectomized untreated; (3) 8 thyroidectomized and castrated; (4) 7 thyroidectomized-castrated rats treated with 1.25 mg. testosterone propionate; (5) 8 unoperated fed a diet containing 0.2% thiouracil* 3 weeks prior to, and for 4 weeks following the bleeding. The results are depicted graphically in Figure 3.

It will be seen that castration in thy-

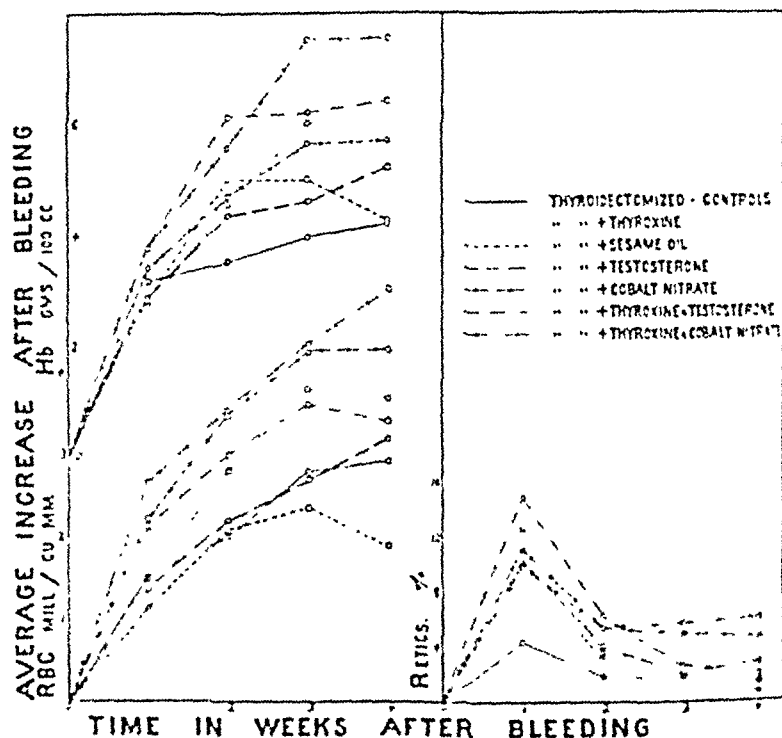


FIG. 2.—A composite of the effects of thyroxine, testosterone, cobalt, thyroxine plus testosterone and thyroxine plus cobalt on average RBC, hemoglobin values and reticulocyte percentages in thyroidectomized rats 4 weeks after bleeding.

5. *Effects of Cobalt and Thyroxine.* This combination proved to be the most powerful stimulant in the series for RBC and hemoglobin regeneration and for high sustained reticulocyte values. The bone marrow were also intensely stimulated and the Hb/c erythrocyte and cell ratio were correspondingly increased in this group (Fig. 7).

No significant trend could be detected in the total and differential WBC counts of any of the groups in this series.

Thyroidectomized rats did not cause a greater delay in RBC regeneration than that produced by thyroidectomy alone. Testosterone, which was only slightly effective in accelerating RBC regeneration in the thyroidectomized rats (Series 2), proved to be a more potent stimulant in the thyroidectomized-castrated rat. Hemoglobin regeneration was more delayed in the thyroidectomized-castrated than in the thyroidectomized animals; this delay was not

* Dr. T. W. Cline, Johns Hopkins Laboratory, Baltimore, Md., provided a generous supply of thiouracil.

come by testosterone injections. Thiouracil treatment inhibited both RBC and hemoglobin regeneration to about the same extent as that noted in thyroidectomized-castrated rats. Normal, thyroidectomized and thyroidectomized-castrated rats all displayed reticulocyte responses of approximately the same magnitude; testosterone treatment increased reticulocyte counts during the 1st week following bleeding but not beyond this period. Thiouracil fed rats possessed the smallest reticulocyte counts at all times during the experi-

been thyroidectomized 6 months previously were divided into 2 groups the day after bleeding. Six unoperated and 5 thyroidectomized rats received daily injections of 0.1 mg. thyroxin and the other 10 served as untreated controls. The results corresponded in general to those obtained in the previous series of experiments. In this group, however, the delay in RBC and hemoglobin regeneration was strikingly apparent only during the 1st week following bleeding. Thyroxin exerted its usual stimulatory influence on

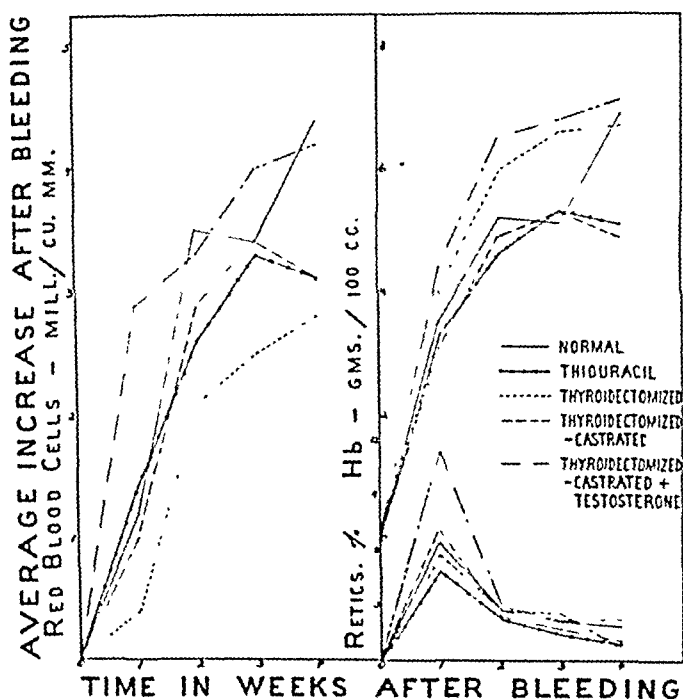


FIG. 3.—Effects of castration and testosterone plus castration on average RBC, hemoglobin values and reticulocyte percentages in thyroidectomized rats following bleeding. The influence of thiouracil on the behavior of bled unoperated rats is also seen.

ment. White cell counts showed no significant alterations in any of the animals except for the thiouracil series in which the usual chronic granulocytopenic state was detected.¹⁰

SERIES 4. In the 3 series described above the thyroidectomized animals were employed 1 to 2 weeks following the operation. An additional experiment was performed to determine the type of result obtainable with long-time thyroidectomized rats. Accordingly 12 rats that had not been operated upon and 9 which had

the RBC, hemoglobin and reticulocyte values but to somewhat different extents in the unoperated and thyroidectomized groups. Again the total and differential white cell counts revealed no significant trends. A moderate tendency to hypoplasia noted in the marrows of the thyroidectomized animals in this series was corrected by the thyroxin treatment.

Discussion. A definite relation between the thyroid gland and the process of erythropoiesis is indicated by the results of the present investigation. Support for this

contention comes from a study of both the peripheral blood picture and the bone marrow in bled animals. Thus thyroidectomy or hypothyroidism induced with thiouracil causes a marked delay in, and thyroxin injections an acceleration of RBC and hemoglobin regeneration in hemorrhagic anemia. The ability of thy-

roxin to stimulate RBC production has also been demonstrated in the hypophysectomized rat.^{25,33}

The erythropoietic effect of thyroxin appears to be more evident in thyroidectomized than in unoperated animals. This may find its explanation in the different amounts of thyroid hormone existing in

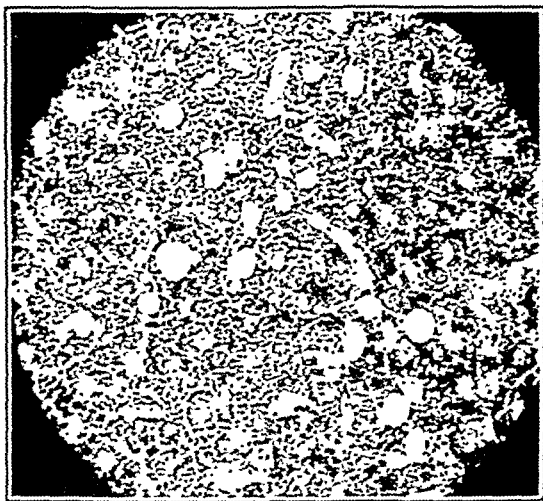


FIG. 4.—Bled rat unoperated upon and given no treatment. Some hyperplasia is still observed 30 days after bleeding. This and following figures represent median sagittal sections of rat femoral bone marrow, fixed in Downey's solution, decalcified in formic acid and sodium citrate, and stained with Kingsley's polychromatic solution. $\times 150$.

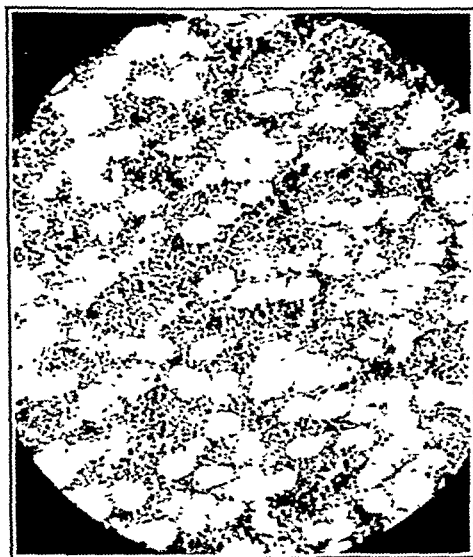


FIG. 5.—Bled thyroidectomized rat given no treatment. Fatty infiltration and slight hypoplasia of erythrogenic elements are apparent 30 days after bleeding (see Fig. 4).

the blood and in the reciprocal effects of this hormone on the anterior hypophyses of such animals. The "paradoxical" decrease in RBC count following an initial polycythemia induced by thyroxin³⁵ may perhaps be similarly explained.

Previous work⁷ has shown that castration in the male delays the regeneration of red cells following bleeding. The present studies indicate, however, that castration

superimposed upon thyroidectomy causes no further inhibition of RBC regeneration. Since testosterone has been shown to be a potent stimulus to erythropoiesis in the hypophysectomized rat³² and in the castrated rat,^{7,29} it was expected that this hormone would augment the action of thyroxin; this, however, did not occur. Moreover, testosterone, by itself, only slightly accelerated RBC regeneration in

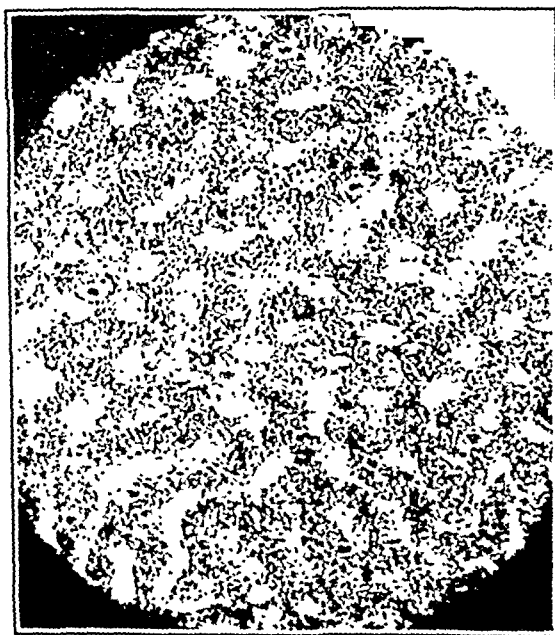


FIG. 6.—Bled thyroidectomized rat treated with thyroxin for 30 days. Considerable repair of the thyroidectomy hypoplasia is observed (see Fig. 5).

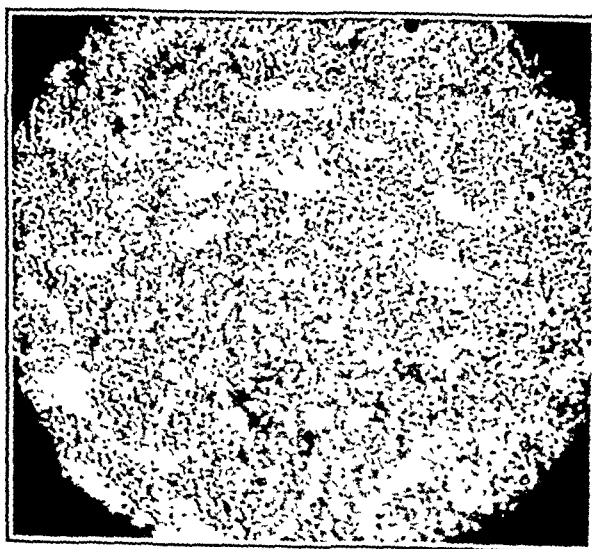


FIG. 7.—Bled thyroidectomized rat treated with thyroxin and cobalt for 30 days. Intense hyperplasia and hyperemia noted; many densely packed erythrocytic areas are visible.

bled thyroidectomized animals. This may have been due, in part, to a sensitivity of the thyroidectomized animal to sesame oil, the vehicle for testosterone. It is more likely, however, that injections of testosterone into thyroidectomized or unoperated animals, in which relatively high levels of endogenous male sex hormone already exist, are not effective as in hypophysectomized or castrated animals in which the male hormone concentration is low. Previous work³³ in addition has shown that daily injections of 2 mg. of testosterone are not as effective as 1 mg. doses. The fact that testosterone exerts a considerably greater effect in accelerating RBC regeneration in the thyroidectomized-castrated than in the thyroidectomized rat would lend additional support to this hypothesis.

The endocrine effects on hemoglobin synthesis are complex. Thyroidectomy inhibits and thyroxin stimulates hemoglobin regeneration in bled rats (the present work). On the other hand, testosterone depresses hemoglobin formation in the normal male rat and castration enhances it.⁷ Difficult to reconcile with this is the fact that castration in the thyroidectomized rat further delays hemoglobin synthesis and testosterone accelerates its formation in the thyroidectomized-castrated rat; likewise the synergism of testosterone and thyroxin in speeding hemoglobin synthesis remains unexplained. It seems possible that, as for red cell numbers, hemoglobin values in endocrine-treated animals are determined by the relative levels of endogenous and exogenous hormones in the blood and the sensitivity of the bone marrow to hormonal stimulation under these conditions.

The results obtained from the peripheral white cell studies were disappointing. No significant trends were observed in any of the animals with the exception of 1 thyroidectomized group which displayed a rise in total white cell count, but even this was transient and soon disappeared. The absence of positive effects on the white cell picture does not necessarily constitute

satisfactory evidence that the regeneration or distribution of these elements is not influenced by thyroid absence or excess. A considerable variation in leukocyte count has been usual among normal non-infected rats of our colony and this would tend to militate against the use of such animals for studies involving relatively small changes in white cell numbers.

Korenchevsky and Hall¹⁷ have asserted that it is not possible to know whether the effects of sex hormones on the blood are due to changes in bone marrow activity or blood volume. They claim that variation in fat content of the marrow, one of the criteria employed by us in this as well as previous studies, "cannot be used as a reliable diagnostic feature of hemopoietic activity." We cannot agree entirely with this statement, for if fat does invade the marrow and replaces blood-forming tissues, the total hemopoietic activity is most likely depressed. Although it may be true, as they have found, that in certain experimental conditions (*e. g.*, after castration) the quantity of marrow fat is proportional to that found distributed throughout the body, this does not hold in every case. For example, it has been shown³¹ that after hypophysectomy the marrow becomes hypoplastic and infiltrated with fat; this extensive vacuolization obviously cannot be explained in terms of an increased fat content of the body following hypophysectomy. In addition it should be emphasized that the fat content of the marrow has not been employed as the sole criterion in interpreting peripheral blood changes. The conclusions that the hormones affect the erythropoietic process itself in previous work^{32,33} and in this report have been based on a combination of several points of evidence, *e. g.*, reticulocyte behavior, marrow histology and cytology, and the general lack of correlation between changes in red cell numbers and hemoglobin concentrations in the peripheral blood.

It is still an open question as to whether the thyroid directly regulates the erythropoietic process or whether this control is

a secondary manifestation of the changes in general metabolism induced by the gland. Excess or diminishing thyroid activity is also associated with changes in pituitary function and these too may exert an influence on erythropoiesis. A true answer to the problem of direct or indirect control of hemopoiesis could possibly be gained by studying the activity of bone marrow in tissue culture under the influence of hormonal agents.

In these studies the combination of thyroxin and cobalt produced the greatest stimulatory influence on red cell and hemoglobin regeneration, reticulocyte numbers and bone marrow activity. Cobalt has also been shown to act synergistically with testosterone in accelerating erythropoiesis.⁷ It is suggested that small amounts of this metal be administered along with the appropriate hormones in the treatment of clinical anemias accompanying endocrine deficiency.

Summary. (1) Thyroidectomy or thiouracil treatment inhibited the return of RBC and hemoglobin to normal levels following bleeding. Studies of leukocyte changes were inconclusive. Injections of thyroxin speeded regeneration of RBC and hemoglobin to a greater degree in bled thyroidectomized than in bled unoperated rats.

2. Castration in thyroidectomized ani-

mals did not further delay RBC regeneration, but caused a greater inhibition of hemoglobin synthesis. Testosterone was a more effective erythropoietic agent in the thyroidectomized-castrated than in the thyroidectomized rat. The combination of testosterone and thyroxin was little more effective than thyroxin alone.

3. Cobalt markedly stimulated RBC and hemoglobin production in bled thyroidectomized rats; the combination of cobalt and thyroxin was even more powerful.

4. The hormones and cobalt, alone or in combination, all evoked marked reticulocytosis during the week following bleeding; but cobalt, and cobalt in combination with thyroxin, were the only treatments which maintained the reticulocytes at high levels throughout the experiment.

5. Neither thyroidectomy nor administration of the hormones or cobalt caused any significant change in the total or differential white cell counts of bled rats.

6. The bone marrow of thyroidectomized rats displayed a slightly greater state of hypoplasia and a lower erythroid/myeloid cell ratio than seen in animals not operated upon 3 to 4 weeks after the bleeding. Thyroxin tended to correct these changes; but cobalt, and especially the combination of cobalt and thyroxin, produced the greatest degree of marrow hyperplasia.

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THE USE OF RADON OINTMENT IN THE TREATMENT OF LATE IRRADIATION ULCERS

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A HALF century ago, Roentgen announced to the world, his epoch-making discovery of the rays that bear his name. It did not take long for the medical profession to learn that their diagnostic and therapeutic possibilities were fraught with a danger which soon tempered its early enthusiastic response, 200 late radiation injuries having been compiled in the first 7 years.¹ While the science of radiology has progressed to the point where it now rests on a firm foundation, only recently have measures been devised which counteract successfully some of the harmful effects of radiation.

There is no essential difference between the reaction caused by Roentgen rays and those effected by radium. The dosage usually is so planned that the maximum safe reaction becomes manifest. If the skin tolerance is exceeded, ulceration and necrosis may occur. The ulcer may heal, but the tissues are atrophic and are susceptible to slight trauma. In such areas, late radiation ulcers sometimes develop. The late changes due to radiation are hyperpigmentation, or loss of pigmentation, sparseness or absence of hair, few or numerous telangiectases, diminished or absent activity of sweat and sebaceous glands and various degrees of skin atrophy, keratoses, late ulceration or cancer.

Histologically, the immediate initial change¹⁰ following irradiation is a focus of local edema and infiltration of leukocytes in the corium, followed by degeneration of the nuclei. The intimal cells of the blood-vessels of the corium and subcutaneous tissues undergo swelling, subsequently causing obliteration of the lumina.

The larger vessels show similar changes, with thickening of the adventitia and media, and partial or complete occlusion. There are areas of varying sizes of necrosis in the corium, with loss of the epidermal appendages. The collagen shows various degrees of homogenization up to extensive fibrosis with the formation of dense sclerotic areas of connective tissue.¹¹ Later, there may be new formation of capillaries arising from the thickened vessels of the upper cutis. Sebaceous glands are destroyed and hair follicles are next in their degree of susceptibility.

Late radiation ulcers occur months to years after radiation has been employed. The ulcers usually follow infection or trauma of a minimal nature to an area showing the stigmata of previous radiation. In some cases, the trauma may be so minimal that the patient does not remember the incident. As a result of a diminished blood supply in the tissue, an ulcer forms which is indolent, usually quite painful and tender. The ulcers may become gangrenous and undergo malignant degeneration.¹ The pain may be so intense that the patient may develop drug addiction and become a total invalid. In addition, the medical profession cannot afford to minimize the medico-legal aspects of the problem.

It long has been known that individuals vary in their susceptibility to radiation⁹ and that different regions of the body manifest different responses to an erythema dose.⁵ The aged are usually less sensitive than infants, whereas females are usually slightly more sensitive than males. A coarse skin or anemic skin will react

less rapidly than thin skin or skin possessing good color. Blondes are usually more susceptible than brunettes, and Negroes are most resistant. The scalp is the least sensitive area, while the face is probably the most sensitive. The extensor surfaces are more resistant than the flexor surfaces, while the flexures such as the axilla and groin are very sensitive. Chemical irritants enhance the irradiation effect. Finally, a small treatment area will tolerate considerably more irradiation than will a large area.

The initial response of the skin to radiation is not always comparable with the delayed effects. An area of skin which shows a minimal initial susceptibility, as evidenced by the time of appearance and degree of erythema, may undergo late radiation necrosis following a dose that more susceptible areas would tolerate with safety. Thus, relatively ischemic areas may break down, despite exhibition of only minor primary radiation effects. This probably accounts for a certain irreducible percentage of late radiation ulcers.

With the advent of heavily filtered, exceedingly high voltage radiation (1000 kv.) it has been possible to deliver a tumor dose deep in the tissues with less effect on the skin than is obtained with the customary deep voltage (200 kv.) therapy.¹² Despite the refinements in equipment and in technique, however, radiology departments throughout the country continue to see late radiation ulcers, indicating the importance of dosage factors and individual susceptibility.

The toll exerted on the early workers in the profession¹ was the result of ignorance of the harmful possibilities of the new rays. It is therefore somewhat startling that, of 70 cases of radiation injuries recently seen by Uhlmann,¹³ 35 were in physicians. Rather significant is the fact that the radiologists, that section of the medical profession most exposed to irradiation, but at the same time keenly aware of its potential dangers, were represented by only 3 members. Only 30 of the 70 cases received their injuries as a result of

treatment with Roentgen rays and radium, the remainder being injured in the course of diagnostic or technical work. Of the 30 cases, but 14 had received therapy for malignancy. Of 37 cases of late radiation ulcers reviewed by Davis,³ 27 were the result of treatment for benign conditions, 6 were the result of accidental irradiation during fluoroscopic procedures, and 4 resulted from irradiation for malignancy. What better evidence is needed that most radiation injuries are avoidable?

Until recently, these ulcers were notoriously refractory to medical treatment. The previous methods employed were varied, and the good results obtained were the exception rather than the rule. Periarterial sympathectomy, variations of acid-base equilibrium, salt-free diet, cod-liver oil ointment, ultraviolet irradiation and autohemotherapy all experienced temporary popularity, soon to be relegated to the medical limbo. The use of the fresh whole leaf of *Ala vera* gave more encouraging results than any previous non-surgical method of therapy,^{7,16} but it failed to produce the anticipated high percentage of cures. Plastic repair was necessary in many cases as the only means of cure.

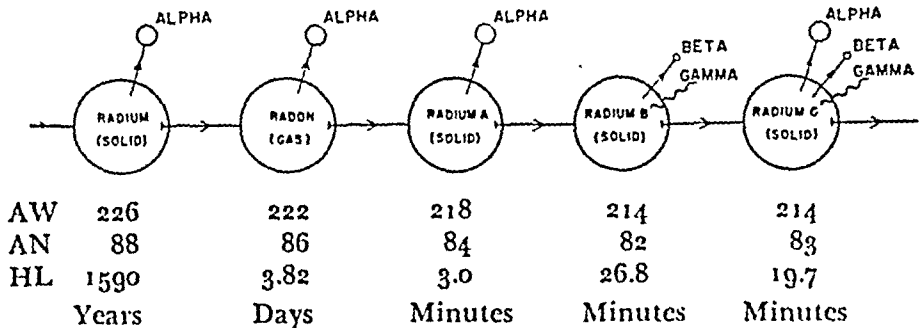
In 1930, Uhlmann first began to use radon ointment in the treatment of late irradiation ulcers and has since that time effectively demonstrated its value as a therapeutic agent.^{13,14,15} The active principle in the radon ointment is its high concentration of alpha particles and to a less extent beta particles and gamma radiation released by the atomic disintegration of the radon gas. Of 70 cases treated in this manner, only 2 were not cured, and these had residual carcinoma in them.

Radon Ointment Preparation. Radon ointment is prepared by dissolving radon under pressure in lanolin. Radon occurs normally in the gaseous state and is given off by radium prepared in the form of an aqueous solution of one of its salts. As the gaseous radon is formed, it bubbles up through the solution of radium and can be collected, purified, concentrated and measured in a closed system.

Radium becomes radon by the spontaneous emission from its nucleus of an alpha particle. Alpha particles are fast traveling nuclei of helium atoms which can be stopped by a sheet of paper, can penetrate tissue to a depth of only 0.030 to 0.050 mm., and are completely stopped by the material in the walls of the ordinary radium containers. Thus, they are not utilized when radium is used therapeutically in closed containers. Eventually, they annex 2 electrons and become helium atoms.

speed electrons which can penetrate tissue to a depth of 15.4 mm., but are stopped, as are the alpha particles, by the walls of the ordinary radium containers now in use. The gamma rays, which are the rays commonly employed in radium therapy, are electromagnetic in nature, with properties similar to light. They correspond to Roentgen rays, but are much shorter in wave length and are much more penetrating, only 50% being absorbed by 13 cm. of tissue.

Commercial radon contains radon, Ra-



Disintegration of radium and its immediate descendants. *AW*, atomic weight; *AN*, atomic number; *HL*, half-life.

FIG. 1—Radon And Its Short Lived Disintegration Products. (Glasser, Quimby, Taylor, Weatherwax: Physical Foundations of Radiology, New York and London, Paul B. Hoeber, Inc., 1914.)

TABLE 1.—THE RADIOACTIVE DECAY OF RADON

Elapsed time	Fraction remaining	Elapsed time	Fraction remaining
0	1.000	3 days	0.581
4 hours	0.970	4 "	0.484
8 "	0.941	5 "	0.404
12 "	0.913	6 "	0.337
16 "	0.886	7 "	0.281
20 "	0.860	8 "	0.235
1 day	0.834	9 "	0.196
2 days	0.696	10 "	0.163

The residual activity after any other time interval can be computed from this table by multiplying the appropriate factors: for example, after 3 days 20 hours the fraction remaining is the product of the values for 3 days and for 20 hours, thus $0.581 \times 0.860 = 0.500$; after 15 days the fraction remaining is the product of the values for 10 days and for 5 days, $0.163 \times 0.404 = 0.066$. (Evans, R. D., Massachusetts Institute of Technology, Cambridge, Mass., in process of publication. Physical Constants of Radon Ointment.)

In less than 4 days, one-half of a given amount of radon will have disintegrated into a new element of lower atomic weight, losing an alpha particle and becoming Radium A. Even more rapidly, Radium A loses an alpha particle and becomes Radium B. This disintegrates in a matter of minutes to Radium C through the spontaneous emission of beta and gamma radiation.

The beta particles produced by the disintegration of Radiums B and C are high

diums A, B and C, and continues to emit alpha, beta and gamma radiation, in unvarying fixed proportions for 30 days. Each day it loses 18% of its strength of the previous day. During the entire period of its activity, 90% of its emissions are alpha particles.

The question will naturally arise in the reader's mind as to why the gamma rays being emitted from the radon ointment will not produce still further damage to

the tissue already injured by overexposure to this type of radiation. Three factors are involved in explaining this apparent therapeutic paradox:

1. The amount of radon incorporated into the ointment is very small. The absolute concentration of gamma radiation emitted from the ointment during an 8 hour application in commonly employed therapeutic strength (200 e.s.u.) is 3.3×10^{-5} mc. hours, as contrasted to 5000 mc. hours commonly employed in the treatment of carcinoma of the cervix.

2. Due to its great penetrability, only a small amount of the gamma radiation is absorbed in the most superficial layers of the treated tissue.

3. Ninety % of the radiation is composed of alpha particles. In the superficial layers of tissue where all the energy from the alpha rays is absorbed, the amount of energy absorbed from the alpha rays is more than 10,000 times that absorbed from the gamma rays.⁴

Thus, the treated area receives an insignificant amount of gamma radiation and a high concentration of alpha radiation.

At the present time, nothing is known concerning the mode of action of radon in promoting the healing of ulcers. It is believed that capillary proliferation is stimulated in the ordinary avascular bed of the ulcer. Griffith⁵ has shown that radon ointment applied to the skin causes an increase in the cutaneous capillary count.

Method of Treatment. When late radiation ulcers are seen for the first time by the radiologists, it is important to determine whether there is any evidence of malignancy. We believe that a biopsy should be performed in any suspicious case. In large ulcers, it may be necessary to perform multiple biopsies of all the areas under suspicion. Contrary to the experience of Uhlmann and Grossman,¹⁵ who believe that a prompt response to radon ointment will tend to exclude the recurrent or residual malignancy, it has been our observation in 2 cases (Cases 7 and 12) that such a diagnostic criterion is not always reliable. It should be remembered that biopsy, per-

formed on heavily irradiated tissue, is often inconclusive, for bizarre, edematous cells in an ulcer, the result of prolonged ischemia, may be mistaken for tumor cells.

Varying degrees of infection are present in most of the ulcers. In the majority, the infection is minimal and requires no adjuvant treatment. In some cases, the presence of an adherent slough or necrotic tissue will act as a mechanical barrier to the penetration of the alpha particles and will require preliminary treatment with zinc peroxide, or sulfanilamide-allantoin ointment for several days. Where these changes are not marked, one may be able to institute the course of radon therapy and apply the measures for combating infection in the intervening periods.

The ointment should be applied as close as possible to the viable tissue. This requires that the ulcer be thoroughly cleansed, debrided and dried. In this manner, the lanolin base has an opportunity to be absorbed into the deeper layers of the tissue and thus increase the depth of effective treatment past the 0.1 mm. range of the alpha particle. Care should be taken not to allow the ointment to come in contact with the operator's hands, the material being applied by means of a wooden tongue blade. It is applied evenly over the lesion, including a periphery of normal tissue, in a thickness of about 1 mm., 1 cc. covering approximately 10 sq. cm. of a lesion. The dressing applied over the ointment must be air-tight and this is achieved by the use of a rubber dam, oil silk, or cellophane which is fastened down with overlapping adhesive strips or scotch tape. In some cases, it may be desirable to apply gauze on top of the air-tight dressing before securing it with adhesive. When there is a pronounced secretion, it may be advisable to reverse the procedure and apply a thin layer of gauze on top of the ointment, followed by the protective dressing and adhesive. Liquid adhesive has been used at the periphery of the protective covering to further seal in the radon gas. The location of the lesions in various irregular contours of the body will often tax the ingenuity of the physician in devising methods of guaranteeing an air-tight dressing. After a specified time, the patient removes the dressing and following superficial cleansing, applies either a bland ointment such as boric acid ointment, or a bactericidal preparation

if deemed advisable. Irritating medications and prolonged exposure to strong sunlight are prohibited.

The dosage employed is a matter of experience. The ointment is obtainable commercially in 4 strengths: 100, 200, 500 and 1000 electrostatic units per cc. These concentrations equal the commercial Alphatron's 40, 80, 200 and 400 μ c. per cc. The radon concentrations also are graded as *mild*, *moderate*, *strong* and *special*, corresponding to the strengths mentioned above. It has been our practice to apply the 200 e.s.u. ointment for 8 hours, once a week. If the response is slow, one may increase the period of application for any period up to 24 hours once a week. In some instances, we have employed two 8 hour applications per week. In some of our early cases, we used 100 e.s.u. but gave this up in favor of the 200 e.s.u. strength. We have never used the 1000 e.s.u. concentration.

Case Abstracts. CASE 1. A white male, aged 70, treated for epithelioma on the dorsum of the right hand; 6670 r were delivered in 6 equal doses on Chaoul apparatus, June 6 to July 11, 1942. The factors were: 45 kv.p. with 0.2 mm. of nickel filtration at 5 cm. T.S.D. through a 3 cm. portal. It was completely healed in January 1943. He scratched his hand in January 1945 with formation of radiation ulcer 3 cm. in diameter. Twenty-five radon ointment treatments were given between Feb. 1, 1945, and May 22, 1945, which healed the ulcer; 200 e.s.u. strength for 24 hours used twice a week.

CASE 2.—A white male, aged 41, treated for epithelioma on the dorsum of the right hand; 1200 mg. hours of radium in contact, filtered with 2 mm. of brass given in July 1930, plus 1550 r in 1 dose at 130 kv.p., 25 cm. T.S.D., with no added filter and 2 cm. portal. It was completely healed in September 1930. It broke down in January 1943; 100 e.s.u. radon ointment applied for 8 hours once a week for 10 weeks. It was completely healed by June 1943. In October 1943, he developed a keratotic horn at the site of ulcer. This finally broke down in October 1944. This second radiation ulcer did not respond to radon ointment and the area was skin grafted with a good result.

CASE 3.—A white male, aged 68, treated for epithelioma on the dorsum of the left hand. The lesion received 8000 r at 25 cm. at 130 kv.p. with 1 mm. of Al filtration

through a 3 cm. portal, between Feb. 14 and Feb. 21, 1939, in 6 equal doses. It was completely healed in May 1939. After trauma to hand in October 1942, an ulcer appeared. Radon ointment applied 60 times during the entire year of 1943 showed no result. After a pathologic fracture of the underlying metacarpal in June 1945, the ulcer healed spontaneously.

CASE 4. A white male, aged 42, treated for eczema. This patient had 1 minute treatments 2 to 3 times a week over a 2 year period from a dermatologist in 1928 and 1929. The exact dosage and factors are unknown. Fissures developed in the skin of the right hand in 1940. Twenty-seven applications of radon ointment 100 e.s.u. strength twice a week for 8 hours showed only a slight improvement.

CASE 5. A white female, aged 49, treated for hyperthyroidism at another hospital. Statement made by physician that 300 r were given to each of 4 portals in 1939; 1 of the 4 10 x 10 cm. portals had a severe reaction. The factors used were: 135 kv.p. at 30 cm. with 4 mm. Al filtration. In 1943, this area showed late irradiation changes and a 2 cm. portion of it ulcerated; 100 e.s.u. radon ointment applied for 8 hours once a week, completely healed it. Another area in this treatment portal broke down 6 weeks later. Similar treatment over a period of 11 weeks healed it.

CASE 6. A white female, aged 43, treated for hyperthyroidism. She received 12,000 r over the anterior neck through a 10 x 13 cm. portal between June 1933 and September 1944 in 400 r doses. The factors were: 135 kv.p. at 30 cm. with 0.25 mm. of copper filtration. Severe later radiation dermatitis developed over the anterior portion of the neck in 1942. This ulcerated in 1945. Application of 200 e.s.u. radon ointment once a week for 8 hours produced marked improvement and 2 months later the ulcer healed, despite the fact that no further radon ointment had been given. Healing was complicated by an urticaria produced by sensitivity of the skin to adhesive tape.

CASE 7. A white male, aged 69, first seen in 1940 with a basal cell carcinoma on the neck which had been present for 12 years and had reached a diameter of 4 cm.; 7500 r were delivered to the lesion by Chaoul therapy through multiple portals to provide uniform distribution of the above dose. The

factors were: 45 kv.p. with 0.2 mm. of nickel at a distance of 3 cm. The lesion healed slowly, the result being thin atrophic skin over a thick fibrous layer bound down to the deep fascia of the neck. It was always nodular. In 1941, a small ulcer developed in it and off and on during the next 2 years there were recurrent ulcerations in this scar that would heal slowly under sulfonamide ointments. In February 1943, 3 ulcers were present in the scar, 1 in the mid-line and 1 to each side of it. Biopsy showed residual basal cell carcinoma in 2 of them. In the next 3 months he received weekly 8 hour applications of 100 e.s.u. radon ointment. In July 1943, all the ulcers were healed. A small ulcer appeared at the site of the previous middle ulcer 3 months later and in the next year gradually enlarged to a diameter of 1.2 cm., despite the use of radon ointment. In September 1945, it measured 2 x 3 cm.

CASE 8. A white male, aged 55, first seen in November 1941 for a 1.3 cm. basal cell carcinoma involving the inner canthus of the right eye. It was treated with 9000 r on the Chaoul apparatus in 22 equal doses. The factors were: 45 kv.p. with 0.2 mm. of nickel at a distance of 3 cm., through a portal 2 cm. in diameter. Fifteen months later, an irradiation ulcer 1.5 cm. in diameter appeared. He had increasing pain for 2 months. At that time, 100 e.s.u. radon ointment was applied for 8 hours weekly for 3 weeks. The lesion was practically healed 1 week after the second application. It has remained healed.

CASE 9. A white male, aged 62, first seen in March 1941, with a 0.75 x 1.5 cm. basal cell carcinoma on the right cheek near the outer canthus. This was treated with 487 mg. hours of radium within 3 days at 1 cm. distance using 1 mm. platinum filtration. This was followed in June 1941 with 9000 r in 28 equal daily doses using 200 kv.p. with 2 Thoreous filtration through a 5 cm. portal at 25 cm. distance. The lesion was entirely healed in July 1941, but it broke down in January 1943. At this time 100 e.s.u. radon ointment was applied for 8 hours weekly for 3 weeks. The lesion was completely epithelialized 1 week after the second treatment. It has remained healed.

CASE 10. A white male, aged 59. In July 1940, he received 6300 r in 4 equal weekly doses on the Chaoul apparatus for

an epithelioma at the base of the right ala nasæ. In August 1940, he received 3900 r in 3 weekly equal doses using 200 kv.p. with 0.5 mm. of copper filtration through a portal 5 cm. in diameter at a distance of 25 cm. The lesion was entirely healed by November 1940. The site ulcerated in January 1942, and he suffered intolerable pain in this area until consulting the authors in December 1942 for a 1 cm. ulceration; 100 e.s.u. radon ointment used for 8 hours once weekly for 5 weeks healed the ulcer. There was little discomfort after the second treatment.

CASE 11. A white male, aged 61. In 1939 this patient was seen with an 8 x 4.5 cm. basal cell epithelioma on the left side of the nose involving the cartilage of the nose; 2500 r in 13 equal daily doses were delivered at 25 cm. at 200 kv.p. with 0.5 cm. filtration. The lesion healed but the patient returned with an extensive recurrence with further invasion of the nasal cartilage in April 1944. He then was given 4300 r on the Chaoul apparatus with a daily dosage of 500 r. At the same time, 1900 mg. hours of interstitial radium therapy was given, using 0.5 mm. platinum needles. The lesion then healed slowly and by January 1945 there was only a 0.5 cm. ulcer left. During the winter cold weather, it broke down rapidly. Repeated biopsy showed no evidence of malignancy. In February 1945, 200 e.s.u. radon ointment was started and 24 weekly applications for 24 hours healed the ulcer entirely.

CASE 12. A white female, aged 56. This patient had carcinoma of the left breast which had developed in 1942. In June 1943, there was extensive skin involvement with a 3 x 4 cm. node in the right upper quadrant that was beginning to ulcerate. Three 20 x 20 portals over the left breast received 1400 r each at 200 kv.p., 0.5 mm. Cu filtration at 50 cm. In April 1944, there was a foul infected ulcer in the left pectoral fold 3 cm. in diameter and 4 cm. deep. There was also a cancer encuirasse with nodes in the axilla. After secondary infection was cleaned up with zinc peroxide, 200 e.s.u. radon ointment was applied for 24 hours twice a week for 12 weeks. A senior medical student became interested and spent an hour daily dressing patient. Ulcer almost completely healed.

CASE 13. A white male, aged 60. In September 1942, he received 3200 r to a 3 x 6 cm. portal on the anterior thigh. There were 6 equal doses delivered 48 hours apart. In December 1943, an ulcer developed. This was treated with 100 e.s.u. radon ointment for 8 hours once a week for 19 weeks and resulted in complete healing. The factors were: 200 kv.p. at 15 cm. with 2 Thoreous filtration.

CASE 14. A white male, aged 50. Experimental irradiation. In August 1942, this patient received 3465 r in a period of 15 days. There were 6 treatments varying in intensity from 230 r to 924 r; 200 kv.p. was used at a distance of 15 cm. with a 2 Thoreous filter to a 3 x 6 cm. field. An ulcer developed in March 1944; 200 e.s.u. radon ointment applied twice weekly for 12 to 24 hours over a period of 25 weeks healed the ulcer completely.

CASE 15. A white male, aged 49. For a neurogenic sarcoma over the internal malleolus of the right leg, the patient received in 1932, 6000 r in 8 equal treatments over a period of 8 months, 135 kv.p. 30 cm. T.S.D. 1 mm. of Al. Recurrence in 1935 was removed surgically. Radiation ulcer developed in 1938. This healed slowly and spontaneously in the next 2 years. He developed a fungus infection over this site in 1943 in New Guinea and a radiation ulcer soon developed. Treatment was started in April 1945 with 200 e.s.u. radon ointment. It was applied for 48 hours twice a week for 1 month. The ulcer was completely healed at the end of that time.

CASE 16. A white male, aged 58. In 1937 and 1938, the patient treated himself twice a week on a low voltage therapy tube for dermatophytosis of the feet. No idea as to dosage is known. In 1944, he developed extensive ulceration over the lateral border of the right foot. Sixteen bi-weekly treatments with 200 e.s.u. radon ointment for 24 hours were entirely ineffective. Skin grafting is to be done.

CASE 17. A white female, aged 35. This patient had a full dose of superficial Roentgen ray therapy for a plantar wart in 1940. When the plantar wart recurred, another series of Roentgen ray therapy was given. The resulting ulcer never healed and in May 1943, when treatment was started, there was a 0.5 x 1.5 cm. ulcer on the sole of the foot. Twenty weekly treatments

with 100 e.s.u. radon ointment applied for 8 hours were a failure. Complete excision and skin grafting was done in November 1943, with a good result.

Results of Treatment. From January 1943 until August 1945, 17 cases of late radiation necrosis were treated with radon ointment in the Department of Radiology of this hospital. One of these patients (Case 2) had a recurrence of his ulceration 18 months later, while another patient (Case 5) developed a second ulcer in another previously irradiated area. Of the 19 ulcers thus treated, 12 healed completely, 2 showed a good response but failed to heal completely, while 5 showed little or no response. The 2 cases (Cases 7 and 12) which showed a good response but failed to heal completely were known to have proven carcinoma remaining in the lesion when therapy was instituted. Both had inoperable carcinoma that had been subjected previously to heavy irradiation.

Discussion. Our favorable results with this method of treatment, while not as uniformly successful as those achieved by Uhlmann,^{13,14} lead us to believe that his introduction of alpha particle therapy is a new approach and a definite advance in the non-surgical treatment of what has heretofore been a very discouraging clinical problem. While our experience with the problem has been quite limited compared to Uhlmann's, we have been able to follow our cases quite carefully and to satisfy ourselves that radon ointment shows promise of occupying a definite place in our therapeutic armamentarium.

One of the most striking features in its use was the prompt relief of pain when combined with methods for control of the concomitant infection. The change in the mental outlook of the patient was most gratifying to the observer. Definite evidence of healing has been noted in some 1 week after institution of treatment. Such early response augurs for rapid and complete recovery, and the repair process, once begun, may continue even though the radon ointment is no longer applied.

TABLE 2.—ANALYSIS OF DATA

Case No.	Underlying condition	Site of ulcer	Radon ointment applications* 24 hrs. per treatment	Response	Remarks
1	Epithelioma 6670 r (June 1942)	Dorsum of hand (January 1943)	200 e.s.u. twice a week for 12½ wks.— 24 hrs. per treatment	Healed in 10 wks.	Ulcer 3 cm. in diameter Keratotic horn followed healing of first ulcer; 10 mos. later another ulcer developed
2	Epithelioma 1200 mg. hrs. contact radium, 2 min. brass, 1550 r (July 1930)	Dorsum of hand (October 1944)	(a) 100 e.s.u. for 8 hrs., once a week for 10 wks. (b) 200 e.s.u. for 24 hrs., twice a week for 20 wks.	(a) Healed in 10 wks. (b) None	Radiation necrosis and fracture of metacarpal at base of ulcer; granu- lations appeared after fracture
3	Epithelioma 8000 r (February 1939)	Dorsum of hand (October 1942)	100 e.s.u. for 8 hrs., once a week for 1 yr.	Slight	1.5 cm. ulceration, second ulcer ap- peared 6 wks. later, following scratching
4	Eczema Dose unknown (1928 and 1929)	Dorsum of finger (1940)	100 e.s.u. for 8 hrs., once a week for 5 wks.	(a) Healed in 5 wks.	Complicated by urticaria in treated area; this was shown to be due to extraneous factors
5	Hypothyroidism Dose unknown (1939)	Neck (1943)	(a) 100 e.s.u. for 8 hrs., once a week for 11 wks. (b) 100 e.s.u. for 8 hrs., once a week for 8 wks.	Healed in 12 wks.	3 ulcers—2 with residual malignancy all healed at first but 2 with residual malignancy recurred
6	Hypothyroidism 12,000 r (1933 to 1934)	Neck (March 1945)	Sulfonamide ointment 100 e.s.u. for 8 hrs., once a week for 15 wks.—repeated 6 mos. later for 100 e.s.u. for 8 hrs., once a week for 3 wks.	Good at first; no re- sponse later	Ulcer, 1.5 cm. in diameter
7	Basal cell carcinoma 7500 r (1940)	Neck (1941) (February 1943) (October 1943) Inner canthus of eye (February 1943) Outer canthus of eye (January 1943)	100 e.s.u. for 8 hrs., once a week for 5 wks.	Healed in 3 wks.	Ulcer 1 cm. in diameter
8	Epithelioma 6000 r (November 1941)	Upper lip (January 1942)	100 e.s.u. for 24 hrs., once a week for 5 wks.	Healed in 6 wks.	Ulcer 0.75 x 1 cm.; patient not seen until December 1942
9	Basal cell carcinoma 487 mg. hrs. radium (March 1941) 9000 r (June 1941)	Nose and cheek (January 1945)	200 e.s.u. for 24 hrs., twice a week for 24 wks.	Good	Residual carcinoma present; large deep ulcer almost completely filled in, due to treatment
10	Epithelioma 6300 r (July 1940), 3000 r (Aug- ust 1940)	Breast (April 1944)	200 e.s.u. for 24 hrs., twice a week for 12 wks.	Healed in 20 wks.	Ulcer, 1 x 2 cm.
11	Epithelioma 2500 r (January 1939), 4300 r (April 1944), 1800 mg. hrs. (August 1944)	Anterior thigh (December 1943) Anterior thigh (March 1944) Leg (1943) Foot, internal border (1944) Foot, plantar surface (May 1943)	100 e.s.u. for 8 hrs., once a week for 19 wks. 200 e.s.u. for 12 to 24 hrs., twice a week for 25 weeks 200 e.s.u. for 48 hrs., twice a week for 4 wks. 200 e.s.u. for 24 hrs., twice a week for 8 wks. 100 e.s.u. for 8 hrs., once a week for 20 wks.	Healed in 20 wks. Healed in 20 wks. Healed in 6 wks. None None	Ulcer, 1 x 1.5 cm.; patient not seen until April 1945
12	Carcinoma 1400 r to each of 3 x 20 x 20 por- tals (June 1943)	Experimental irradiation (September 1942)			Subsequently removed surgically
13	Experimental irradiation 3200 r (September 1942)				
14	Experimental irradiation 3500 r (August 1942)				
15	Neurogenic sarcoma 6000 r (1932)				
16	Dermatophytosis (1937-1938)				
17	Plantar wart Dose unknown (1940)				

* 100 e.s.u. = 40 microcuries.

200 e.s.u. = 80 microcuries.

In some instances, however (Cases 11 and 14), persistence over a long period of time was rewarded by disappearance of the ulcer. A favorable response to therapy is manifested by increased vascularity in the previously avascular ulcer bed where slight trauma, such as removing fibrinous exudate, will produce bleeding. One may observe the appearance of small capillaries or exuberant granulations at the periphery of the ulcer. In some cases, however, the changes are quite minimal and the repair process is insidious. In 2 of our cases (Cases 5 and 6) complaint of pruritus was soon followed by healing.

Apparently any area of the body is amenable to treatment. It should be noted that most of our failures have occurred on the hands and on the feet. This may be related to the question of diminished blood supply, vascular stasis, lack of adequate connective tissue stroma, or possibly neurotrophic factors.

Meticulous care in the control of infection, eradication of slough, and the cleansing and dressing of a wound may yield gratifying results in what appears at first glance to be a hopeless situation. Case 12 had been heavily irradiated elsewhere, for what was an inoperable carcinoma of the breast. She subsequently developed a deep indolent foul-smelling ulcer in the anterior axillary fold. The surgeons refused to perform a palliative simple mastectomy because of the ulcer, and she

was referred to our department for treatment. For a period of about 4 weeks she received daily applications of zinc peroxide paste, followed subsequently by sulfallantomide ointment. At the time of her death, due to metastases, the ulcer had almost completely healed, despite the presence of residual malignancy in the breast.

While we have encountered no untoward effects from the treatment with radon ointment, its potential dangers should be appreciated, both for the physician and the patient. On testing normal individuals, it has been noted that a definite skin erythema was produced in 8 hours in some cases.⁸ Thus, it behooves the physician to avoid contact with a material to which he may be exposed many weeks in the year. When not in use, the material should be stored under lead protection for detectable gamma radiation has been observed by us by the use of appropriate instruments such as the Geiger counter and the Victoreen minometer.

Summary. 1. Seventeen cases presenting 19 late irradiation ulcers were treated with a new therapeutic agent—radon ointment.

2. Twelve of the ulcerations showed complete healing in a period ranging from 3 to 50 weekly applications.

3. Two lesions having residual malignancy showed a good response but failed to heal completely.

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ADDISON'S DISEASE IN THE NEGRO

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FROM the very small number of case reports which can be found in the literature one gains the impression that the occurrence of Addison's disease in the Negro is extremely rare. This conclusion is somewhat surprising since the incidence of tuberculosis (one of the major causes of destruction of the adrenal glands) is greater in Negroes than in whites. That one is not justified in using the total number of cases reported in the literature as an index of the rarity of a disease seems obvious. If one assumes that the occurrence of Addison's disease in the Negro is far greater than would be indicated by the 21 cases reported in medical literature of the world since 1907, one may speculate: (1) that many cases so diagnosed have seemed to be of insufficient interest to warrant their being reported; (2) that physicians who make the diagnosis are unaware of the paucity of reported cases; or (3) that the disease appears to be rare in Negroes because the diagnosis is usually missed. We believe that all 3 have contributed, but that the third is the reason of greatest importance. It is easily understandable that when dealing with Negro patients the possibility of Addison's disease would suggest itself less frequently than in the case of whites, since pigmentation of the skin is usually one of the most striking manifestations of the disease. Because we feel that it is timely that this problem be brought to the attention of the practicing physician we are adding 5 of our own cases to those already reported.

The first case of Addison's disease in a Negro was reported by Scheult,⁵ in 1907. Lisa, Solomon and Gordon² reviewed the

literature on this subject in 1942 and found 17 reported cases. They added 3 of their own. From then to the present time no additional cases have been described, but Shipley⁷ in reporting upon the therapeutic efficacy of pellets of desoxycorticosterone acetate mentions that 1 patient in his group was a Negro.

Our group consists of 3 patients recently seen by us on whom the clinical diagnosis was made, and of 2 on whom the diagnosis was made at postmortem examination some years ago (the clinical diagnosis was suspected in 1 of these).

Case Reports. Recognized Clinically.

CASE 1. J. R., a 42 year old male Negro, was admitted to the University Hospital on Oct. 7, 1943, complaining of severe weakness, anorexia, nausea and vomiting, chronic cough and pain in the right shoulder. These symptoms had begun 2 years previously and had been gradually progressive. Over this period the patient had lost 70 pounds of body weight (200 to 130 pounds). He stated he had noticed increased pigmentation over the dorsum of the hands, the elbows and the nipples. On the initial examination he was found to be drowsy and lethargic. The blood pressure in the supine position was 80/58. There were large irregular patches of pigmentation on the dorsum of the tongue (up to 1.5 cm. in diameter). Marked pigmentation of the gums, palate and buccal mucosa was present.

Laboratory studies revealed a plasma NaCl of 403 mg. %, N.P.N. 45.5 mg. %, fasting blood sugar 52 mg. %, and a water test⁴ markedly positive in both phases. The urine was negative and there was a moderate hypochromic anemia.

A good rise in blood pressure and gain in strength followed 3 days of treatment with desoxycorticosterone acetate (D.C.A.) and

NaCl. On the 5th day he developed chills and fever with clinical and Roentgen ray evidence of a right basal pneumonitis. Lethargy, asthenia and hypotension returned. Recovery followed intensive therapy with saline, glucose and adrenal cortical extract intravenously; D.C.A. and adrenal cortical extract intramuscularly; and sulfadiazine orally. He was discharged on 3 mg. D.C.A. daily, apparently well (B.P. 115/80), having gained 11 pounds in weight.

On 2 subsequent occasions he has been hospitalized for the treatment of infection (upper respiratory infection in 1944 and osteomyelitis of a finger in 1945). On each of these admissions the findings of acute adrenal cortical insufficiency were present and responded to adequate therapy.

Repeated sputum concentrates and cultures have been negative for acid-fast organisms. The Congo red test was negative.

At present the patient weighs 197 pounds and is doing full time light work without undue fatigue. His blood pressure has been stabilized at about 135/80 with the use of 3 mg. of D.C.A. daily. He takes a high carbohydrate diet (without salt supplement) which includes a late evening feeding.

CASE 2.—E. H., a 50 year old male Negro, was admitted to the University Hospital on Oct. 20, 1944, complaining of weakness and vomiting. His symptoms had begun about 1 year before, during which interval he had lost 45 pounds in weight. For 1 month prior to admission his asthenia had progressed to the point of virtual complete prostration. Both he and his family volunteered the information that his skin had become darker over the past few months.

Examination revealed a man who appeared much older than his stated age and who spoke in a very feeble voice. The blood pressure was 80/50. The skin was that of a dark Negro. There was marked pigmentation of the mucous membranes of the mouth. Large, irregular patches of pigmentation covered most of the dorsal surface of the tongue.

Laboratory studies showed a plasma NaCl level of 485 mg. %, N.P.N. of 41.5 mg. %, 17-ketosteroid excretion of 3.1 mg. per day and a water test⁴ markedly positive in both phases. Chest Roentgen ray showed no evidence of active pulmonary disease. On admission, the blood showed a hemoglobin of 113% with 5.9 million R.B.C. per c.mm.

After hydration these levels were found to be 89% and 4.6 millions respectively. The urine was essentially negative.

After 4 days of treatment with desoxycorticosterone acetate and salt, appetite began to return and the blood pressure stood at 125/80. There was a gratifying symptomatic response. He was discharged after 31 days of hospitalization feeling well and strong, having gained 10 pounds in weight (without evidence of edema) with a B.P. of 120-130/70-80, and taking a daily maintenance dose of D.C.A. When he had failed to return for reexamination 2 months after discharge, it was discovered that he had died in another city. On discharge, he had not been given sufficient D.C.A. to last for 2 months. No autopsy had been done and little information could be obtained regarding the circumstances of his death.

CASE 3.—V. M., a 36 year old Negress, was admitted to the University Hospital on Sept. 10, 1942, with complaints of weakness, weight loss, anorexia, nausea and backache. These symptoms had begun in mild form 3 years previously and had gradually progressed. Weight had dropped from 189 to 115 pounds over this period. A hysterectomy had been done elsewhere 1 year before admission. The weakness and gastro-intestinal symptoms had increased markedly in severity since her operation.

Examination revealed an emaciated, dehydrated Negress who showed evidence of chronic illness. The blood pressure was 100/65. No other physical abnormalities were observed. Complete gastro-intestinal Roentgen rays showed 10% gastric retention in 6 hours. Gastroscopy revealed only atrophic gastritis. Chest Roentgen ray was negative. Pelvic examination was likewise negative. There was a moderate hypochromic anemia. Urine was negative. The water test⁴ was strongly positive in both phases. A persistent daily elevation of temperature to 100° to 101° F. was present throughout the hospital stay. This was associated with a white blood cell count varying from 6000 to 10,000 per c.mm. Miliary tuberculosis was suspected but not confirmed clinically.

The patient remained in the hospital for only 9 days of therapy for adrenal cortical insufficiency. Although a rise of blood pressure to 120/80 occurred, clinical improvement was not impressive. The lack of a

more rapid response to specific therapy was believed to have been due to the presence of toxicity, the cause of which remained obscure. She was instructed in the use of D.C.A. (5 mg. intramuscularly daily), 3 gm. of supplementary NaCl daily and a high carbohydrate diet. It is doubtful that she followed instructions.

One month later she died at another hospital. It was the impression of the attending physician that she died in Addisonian crisis. *Autopsy* was not performed.

Diagnosed Pathologically. CASE 4. F. L., a 22 year old male Negro, admitted on the Dermatological Service of the University Hospital on Nov. 20, 1924. Died on December 23.

Clinical diagnoses: sacrofuloderma, pulmonary tuberculosis and latent syphilis.

Pathologic diagnoses: generalized amyloidosis, with virtually complete replacement of adrenal cortices by amyloid. Tuberculous osteomyelitis of vertebræ (Pott's disease with psoas abscess). Disseminated miliary tubercles in lymph nodes. Serofuloderma. "Addison's disease."

CASE 5. J. C., 60 year old male Negro, admitted on the Medical Service of the University Hospital in a moribund condition on Dec. 5, 1925. Died the following day.

Clinical diagnosis: miliary tuberculosis, Pott's disease, chronic fibroid pulmonary tuberculosis.

Pathologic diagnosis: "Addison's disease." Bilateral chronic caseating tuberculosis of adrenals with very little adrenal tissue remaining. Chronic pulmonary tuberculosis. Generalized miliary tuberculosis (pleura, peritoneum, lungs, heart muscle, liver, spleen, kidneys and lymph nodes). Tuberculous necrosis of third, ninth, tenth and eleventh thoracic vertebræ. Pott's disease.

Discussion. Although changes in the degree of pigmentation of the skin may be difficult to evaluate in some Negroes, the triad of asthenia, gastro-intestinal symptoms (anorexia, nausea, vomiting and diarrhea) and hypotension should suggest the possibility of adrenal cortical insufficiency. Having thought of this possibility one may be aided further by inquiring about any recent increase in the degree of pigmentation of the skin. Two of our most recent patients volunteered the informa-

tion that their skins had definitely become darker in recent months, particularly over the forehead, the dorsum of the hands, the elbows and about the nipples. In 1 case this had been noted by the family before the patient was admitted for treatment. Since patchy pigmentation of the buccal mucous membranes occurs physiologically in a large proportion of Negroes, this sign (an important supplementary criterion in whites) is of little significance. We have been impressed, however, by the presence of large areas of pigmentation on the dorsal surface of the tongue. While Monash³ reports that some degree of pigmentation on the dorsal surface of the tongue may be found in 33% of normal Negroes, the intensity of this finding in the 2 patients who had noted increased pigmentation of the skin, tempts us to relate it to the adrenal insufficiency. Sodeman⁸ and his staff at Charity Hospital in New Orleans where 55% of admissions are Negroes inform us that in their experience obvious pigmentation on the dorsum of the tongue of normal Negroes is extremely rare.

Incidence of Addison's Disease in Negroes. There are several points of interest which militate against the apparent rarity of Addison's disease in the Negro race. Thorn¹⁰ and Blänkenhorn¹ have seen a number of cases which have not been reported. Both feel that the majority of cases go unrecognized. Eight unreported cases have been diagnosed at Charity Hospital.⁸ Sturgis has seen at least 1 unreported case.⁹ It is clear that a thorough survey designed to disclose unreported cases would, relatively speaking, skyrocket the total number of cases which have been recognized; this despite the fact that the majority have undoubtedly gone unrecognized.

The problem of clinical *versus* pathologic diagnoses throws additional light on the question of the difficulty of clinical recognition. Sodeman reports⁸ that of 4 cases in which there was no doubt of the final diagnosis; 3 were diagnosed at autopsy and only 1 was recognized clini-

cally. Of the 21 cases found in the literature, 12 were diagnosed clinically and 9 were discovered at postmortem examination. In the 12 cases diagnosed clinically, the average duration of symptoms before diagnosis was 16.8 months, the average duration of life after diagnosis being 2.3 months. It should be suggested parenthetically, that the relatively good showing of clinical *versus* pathologic diagnoses as determined from the literature may be more apparent than real. Failures in diagnosis are less likely to be reported than successful diagnoses. Of our own group of 5 cases, 3 were diagnosed clinically and 2 at autopsy. Thus, the high percentage of initial diagnoses made at postmortem means that we are missing the clinical diagnosis too frequently. How many times such an undiagnosed Negro

suggest, at least, that Addison's disease is as common in the Negro as in the white race.

Sex Incidence. The total incidence of well-grounded diagnoses of Addison's disease of all races in the 420,500 registrations at the University Hospital from 1925 to 1944 is 0.019%. Of the 78 cases in all races 59% were males and 41% were females. Among the 26 cases in Negroes reported in the literature and by us, 65% have been in males and 35% in females.

Etiology of Addison's Disease in the Negro. Over the past 10 years an impressive change has occurred in the etiologic incidence of Addison's disease in the white race. Whereas tuberculosis of the adrenals formerly comprised 80 to 90% of all cases examined pathologically, the diagnosis of idiopathic adrenal cortical

TABLE 1

All cases autopsied	No. cases	Tbc.	Cortical atrophy	Amyloidosis	Syphilis
U. of M., 1900-1934 (all races)	17	11 (65%)	2 (12%)	3 (17%)	1 (6%)
U. of M., 1935-1945 (all races)	11	6 (55%)	5 (45%)	0	0
Negroes (entire literature), 1907-1945	20	19 (95%)	0*	1 (5%)	0
				U. of M.	

* We are informed that 1 unreported case seen recently by Sheldon and Howes⁶ showed idiopathic cortical atrophy at postmortem examination. This occurred in a young male Negro soldier in the European Theatre of Operations.

patient fails to come to postmortem examination must remain purely speculative, but whatever the number is, it adds to our clinical failures and to the total number of unsuspected cases. There may be some hope in the fact that of the 21 cases reported in the literature from 1907 to 1944, 11 have been reported in the last 8 years.

Of 78 cases of Addison's disease observed at the University Hospital, the 5 reported above have occurred in Negroes (a ratio of 15.6 white cases to 1 Negro case). Of the total registration figures at this hospital, about 1.7% are Negroes. Thus the ratio of whites to Negroes is 58.8 to 1. If these figures were to be taken seriously, they would mean that Addison's disease occurs about 4 times more frequently in Negroes than in whites, registered at this hospital. The figures

atrophy has gradually increased in recent years so that it has now assumed the No. 1 position among the various causes of adrenal insufficiency, being variously reported as occurring in 40 to 60% of all cases. Our own experience in this regard is summarized in Table 1 and is compared with data now available in the literature with regard to Negroes.

Of particular interest is the highly significant fact that of 7 cases of idiopathic adrenal cortical atrophy observed in whites at the University Hospital over a period of 45 years, 5 have occurred over the past 10 year period. Speculation in this connection is beyond the purpose of this report.

That *all* of the cases heretofore reported in Negroes have been due to tuberculosis is, at least, somewhat surprising.

Summary. Five cases of Addison's disease in Negroes are described. This brings the total number of reported cases to 26. There is undoubtedly a much greater number of recognized cases which have not been reported. Probably the largest increment of cases of Addison's disease in Negroes is not being recognized as such. It is extremely doubtful that the incidence of Addison's disease in Negroes is smaller than in whites. Analysis of our own cases leads to the opposite conclusion.

Increased pigmentation of the skin, the most striking hint that the physician may be dealing with Addison's disease in whites, is frequently of little or no value in suggesting this possibility as he examines the Negro patient. If, however, he does think of this possibility, retrospective information from the patient with regard to increased pigmentation of the skin may be helpful. In addition to the usual cardinal signs and symptoms of adrenal

insufficiency marked pigmentation on the dorsal surface of the tongue may be a significant finding.

All of the cases reported in the literature upon which a pathologic examination was made were found to be due to tuberculosis of the adrenals. One of our cases was due to amyloidosis of the adrenals giving a total etiologic incidence on all 20 autopsied cases of 95% for tuberculosis and 5% for amyloidosis. From the information at hand it would appear that idiopathic atrophy of the adrenal cortices (which now accounts for about 50% of all cases in whites) is either non-existent or extremely rare in the Negro. This is probably not a valid conclusion.

Conclusion. Physicians must be on guard for the recognition of Addison's disease in the Negro, which is in all probability much commoner than the number of reported cases would indicate.

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USE OF AN AGGLUTINATION INHIBITION TEST IN STUDYING THE EFFECTS OF VACCINATION AGAINST INFLUENZA

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A READILY available method for measuring influenzal antibodies is based upon the inhibition by convalescent or immune sera of the agglutination of chicken and certain other red blood cells in the presence of influenza viruses. A modification of Hirst's⁹ original agglutination-inhibition (A-I) technique was described by one of us 2 years ago, together with an account of its use in studying an epidemic due to influenza Type A.⁶ The present communication is to report some observations made with this test following vaccination against influenza Types A and B.

Attempts have been made to produce active immunity to influenza with specific vaccines ever since ready sources of these viruses became available.^{6,10,19} Although the early results were not very satisfactory, they tended to indicate that height of specific antibody level and protective immunity were proportional, and that the more virus present in a vaccine the better it was as an immunizing agent. The most promising one yet to be made available on a large scale was a concentrated preparation containing 2 Type A strains (PR-8 and Weiss) and 1 Type B (Lee). The viruses were concentrated from allantoic fluids of embryonated eggs and killed with formalin. This vaccine was studied extensively by the Influenza Commission of the United States Army during 1943 and 1944.^{4,7,8,11,12,14,17} Over 6000 students were vaccinated at 8 colleges scattered throughout the country. An equal number at each of these institutions was studied as controls. The antibody response to the vaccine was comparable to that produced

by natural infection and almost all those vaccinated developed an increase in A-I antibodies for both PR-8 and Lee. Individuals with higher pre-vaccination titers tended to show relatively smaller increases. Peak titers were reached by 2 to 3 weeks. When sera were obtained and re-tested after 3 to 4 months, a 15 to 30% fall in antibody level was noted. In a subsequent Type A epidemic approximately 2.5 times as many cases occurred among the controls as among the vaccinated, an incidence of 8.3% in contrast to 3.3%. The poorest results were obtained when the vaccine was given about 6 weeks before the start of the epidemic,⁴ then 4% of those vaccinated and 6% of the controls developed influenza. The best results were shown when the vaccine was not given until after the epidemic had already started. At one such institution¹¹ only 1.7% of those vaccinated became ill in contrast to 8% of the controls. There was definite protection by the end of 1 week.

In the fall of 1945, in the face of a rising incidence of influenza, mostly due to a Type B virus, the Army directed that this vaccine be administered to all of its personnel. Although the subsequent rapid demobilization of military installations interfered with original plans for a large scale coöperative study of this mass immunization by this and 2 other laboratories in this area, it was possible for us to follow a local group carefully and to carry out the studies which are the subject of this report.

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Materials and Methods. *Study Group.* Adequate information and sera were available from 89 individuals. Of these, 30 were members of this laboratory staff and the remaining 59 were corpsmen at a nearby Army hospital. Their average age was 20 to 30 years.

Vaccine. Influenza Virus Vaccine Types A and B. Refined and Concentrated (Led-erle—Lot No. 2031-27A) was used. Each subject was given 1 ml. subcutaneously in the deltoid region.

Sera. Blood specimens were collected at the time of vaccination and again after 1 and 3 weeks. Each serum was separated and withdrawn shortly after collection and stored in the refrigerator in vials containing sulfanilamide as a bacteriostatic agent.

Agglutination-Inhibition Test (A-I) Test. The modified Hirst A-I technique reported earlier from this laboratory⁵ was used throughout. All specimens from an individual were tested at the same time. Known positive sera were always included. If the titers obtained with these controls differed by more than one dilution, the tests were repeated. Only 4-fold or greater rises in antibody were considered significant. The PR-8, Weiss and Olson (Type A) strains and the Lee (Type B) strain were obtained from Dr. M. D. Eaton (State Virus Laboratory, Berkeley, California). The Saha (Type B) strain was received from Lt. M. M. Sigel (5th Service Command Laboratory, Fort Benjamin Harrison, Indiana).

Records. At the time of vaccination each subject was questioned in regard to recent influenza, previous respiratory infections, and allergy to egg protein. The members of the laboratory staff who were in the study were interviewed 24 and 72 hours after vaccination. The others were questioned in regard to reactions to the vaccine at the time the 1 week post-vaccination specimen of serum was collected. The subjects on the laboratory staff were instructed to report to one of us with all subsequent illnesses. In those instances which clinically resembled influenza, throat washings, and acute and convalescent phase sera were collected for study. Approximately 12 to 14 weeks later follow-up interviews were held with slightly more than half of the entire group.

Plan of Study and Results. *Relation of Type of Reaction to Vaccine to Pre-vaccina-*

tion A-I Antibody Level. Beveridge and Burnet¹ while studying the effects of intradermal inoculation of influenza viruses observed that allantoic fluids infected with influenza virus A or B produced a cutaneous reaction in most adults and in some children. In these children it was usually associated with the corresponding type of circulating antibody. This suggested to us that reactions following the subcutaneous administration of influenza vaccine might be similarly related to already present antibody. To investigate this possibility, the reactions experienced by our subjects were analyzed in terms of each individual's pre-vaccination titer of specific A-I antibody.

Approximately two-thirds of the study group complained of an untoward reaction within 4 to 24 hours following vaccination. Although the kinds of reactions experienced were similar to those previously reported with this vaccine, their distribution was quite different. Fifteen (17%) developed only local reactions; 34 (38%) complained only of systemic symptoms ranging from transient malaise to fever lasting 6 to 24 hours; and 9 (10%) had both local and systemic signs and symptoms. None required hospitalization. Thirty-one (35%) did not complain of any reaction. We followed the classification used by Eaton and Meiklejohn,⁴ and yet the 17 and 38% (or including combined reactions, the 27 and 48%) of our 89 subjects who experienced local and systemic reactions respectively are very different from the 31 and 23% of their 116 with such complaints. Other observers have reported still different distributions. Rickard, Thigpen and Crowley¹⁴ found that 91% of 550 college students had local and 18% had generalized symptoms. Some of these differences may be a reflection of the manner of questioning the subjects, but it is difficult to account for the majority. It was hoped therefore that serologic analysis would yield pertinent information. In Table 1 there are the results of testing the pre-vaccination sera of the individuals in the group classi-

No.	Pre-vaccination A-I antibody levels	
	I' R 8	I' R 8
1	0.00	0.00
2	0.00	0.00
3	0.00	0.00
4	0.00	0.00
5	0.00	0.00
6	0.00	0.00
7	0.00	0.00
8	0.00	0.00
9	0.00	0.00
10	0.00	0.00
11	0.00	0.00
12	0.00	0.00
13	0.00	0.00
14	0.00	0.00
15	0.00	0.00
16	0.00	0.00
17	0.00	0.00
18	0.00	0.00
19	0.00	0.00
20	0.00	0.00
21	0.00	0.00
22	0.00	0.00
23	0.00	0.00
24	0.00	0.00
25	0.00	0.00
26	0.00	0.00
27	0.00	0.00
28	0.00	0.00
29	0.00	0.00
30	0.00	0.00
31	0.00	0.00
32	0.00	0.00
33	0.00	0.00
34	0.00	0.00
35	0.00	0.00
36	0.00	0.00
37	0.00	0.00
38	0.00	0.00
39	0.00	0.00
40	0.00	0.00
41	0.00	0.00
42	0.00	0.00
43	0.00	0.00
44	0.00	0.00
45	0.00	0.00
46	0.00	0.00
47	0.00	0.00
48	0.00	0.00
49	0.00	0.00
50	0.00	0.00
51	0.00	0.00
52	0.00	0.00
53	0.00	0.00
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67	0.00	0.00
68	0.00	0.00
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92	0.00	0.00
93	0.00	0.00
94	0.00	0.00
95	0.00	0.00
96	0.00	0.00
97	0.00	0.00
98	0.00	0.00
99	0.00	0.00
100	0.00	0.00

Reaction to vaccine		No. persons	Pre-vaccination A-I antibody levels												Changes in antibody level 3 weeks after vaccination																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
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None		31 (35%)	20	11	0	25	6	0	11	4	0	28	6	0	8	1	0	72 (81%)	17 (10%)	0	32 (36%)	57 (61%)	32 (36%)	0	17 (19%)	14 (16%)	3 (3%)	10 (11%)	3 (3%)	2 (2%)	6 (7%)	7 (8%)	2 (2%)	2 (2%)	9 (10%)	4 (4%)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
Local		15 (17%)	6	9	0	0	0	0	11	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

fied according to reaction to the vaccine. Unfortunately, no fundamental differences are apparent except in regard to PR-8 antibody among those who had local complaints. However, there were only 15 individuals in this group. It would seem therefore that except for this one possibility, which must be studied further, there is no relation between an individual's pre-vaccination titer and the likelihood of his experiencing an untoward reaction from the vaccine.

Table 1. Relatively fewer of those who experienced local symptoms developed 4-fold or greater rises in antibody titer for PR-8. However, as already noted, this group had proportionately more individuals with higher initial levels for this virus. These results are therefore compatible with the observation that increase in A-I antibody titer following infection or vaccination tends to be inversely proportional to initial antibody level. Similar consideration of the other groups failed to reveal

TABLE 2.—REPRESENTATIVE VARIATIONS IN ANTIBODY RESPONSE TO VACCINE

	Weeks after vaccination	A			B	
		PR-8*	Weiss	(Olson)	Lee	(Saha)
No. 513	0	<8	<8	<8	16	<8
	1	128	64	16	128	32
	3	1024	512	128	256	128
Profile†	+	+	+	+	+
No. 235	0	16	32	8	16	6
	1	64	64	32	64	32
	3	64	64	32	128	256
Profile	+	—	+	+	+
No. 236	0	<8	<8	<8	<8	<8
	1	8	8	8	8	8
	3	8	32	16	512	128
Profile	—	+	+	+	+
No. 522	0	32	8	8	<8	<8
	1	128	16	8	8	<8
	3	256	64	32	16	8
Profile	+	+	+	+	—
No. 531	0	8	32	8	16	<8
	1	8	32	8	32	8
	3	16	64	16	128	64
Profile	—	—	—	+	+
No. 241	0	32	16	8	16	16
	1	32	32	16	32	32
	3	32	32	16	32	64
Profile	—	—	—	—	+
No. 215	0	8	32	32	16	32
	1	16	64	64	32	32
	3	16	64	64	32	32
Profile	—	—	—	—	—

* Virus strains in italics are those contained in the vaccine.

† "Profile" represents pattern of significant changes in A-I antibody levels produced by vaccination.

Relation of Reaction to Vaccine to Antigenic Stimulation. To investigate the possibility that the capacity for antigenic stimulation by the vaccine might be related to the immediate reaction it elicits in an individual, the changes in A-I antibody 3 weeks after vaccination were compared for each of the groups indicated in

any correlation between reaction and increase in A-I antibody. The reactions of those who at the end of 3 weeks had attained an A-I titer of 1:256 for one or more of the viruses in the vaccine were then analyzed separately. There were 37 individuals with such high levels. Among this group of good antibody pro-

ducers there were 13 (35%) without any reactions, 5 (14%) with local, 15 (40%) with systemic, and 4 (11%) with combined reactions. Although this represents only 42% of the entire study group, the distribution of reactions is strikingly similar to that shown in Table 1.

Relation of Pre-vaccination History to Initial A-I Antibody Levels. In an attempt to find an explanation for the considerable variation in pre-vaccination A-I titers, our data were analyzed in terms of the histories of upper respiratory infections obtained at the time of vaccination. There were 31 who reported having frequent upper respiratory infections and 9 who gave a history of having had influenza during the preceding 2 years. But among the former 31 there were 20 (64%) initial A-I antibody titers of 1:16 or less for PR-S, 25 (81%) for Weiss, and 20 (64%) for Lee. Among the 9 with histories of recent influenza there were 6 with such low levels for PR-S, 6 for Weiss, and 5 for Lee. This distribution is almost identical with that for the study group considered as a whole (Table 1).

Rickard, Thigpen and Adams¹⁵ while studying influenza Type A in 5 infants found that the acute phase A-I titers ranged between 1:26 and 1:79. Since these infants were too young to have been exposed to a previous influenza epidemic, they suggested that the base line titers might represent non-specific inhibiting substance similar to that found in the serums of some normal laboratory animals. It is entirely possible that some of the lower pre-vaccination titers found in our adults were also of this nature. Nevertheless, despite our inability to correlate them with influenza or respiratory infections which the subjects recalled, most of the higher pre-vaccination titers probably still reflected previous clinical or sub-clinical influenzal infections.

Time of Appearance of Antibodies. Since it had been reported^{8,11} that this vaccine gave significant protection by the end of 1 week against infection by a Type A virus, we attempted to determine the time of

appearance of increase in the several A-I antibodies it elicited. There were 86 individuals from whom pre-vaccination, 1 and 3 week post-vaccination serum specimens were available. Analysis of our data suggests that the A-I antibodies for Type A tend to appear earlier than those for Type B. Thirty-two (37%) attained their highest level for a Type A strain in 1 week, while only 21 (24%) did this for the Type B virus. Of those reaching their peak titer in 1 week for only 1 strain there were 17 (20%) for Type A, and 6 (7%) for Type B.

Relation of 3 Week Post-vaccination A-I Titers to Incidence of Subsequent Respiratory Infections. Between 11 and 14 weeks after vaccination it was possible to interview 52 of the group in regard to their general well-being. Thirty-three reported no respiratory illnesses while 19 complained of having had "colds." Eight said that they had had more "colds" than during the preceding 5 years. Yet 11 of the 19 with histories of subsequent respiratory illnesses produced A-I titers of 1:256, or above, for 1 or more of the viruses within 3 weeks after vaccination, while only 9 of the 33 who did not develop upper respiratory infections developed such high antibody levels. Although the majority of these infections was probably not influenza, we did have 2 serologically proven instances due to a Type B virus among the 30 individuals in the laboratory. Both of these illnesses were associated with low grade fever, malaise and leukopenia and were in men who had not produced high levels of B antibody after vaccination. The results of testing their pre-vaccination, post-vaccination, acute and convalescent phase sera against the Lee virus are contrasted in Chart 1 with those of another subject who was sick at about the same time with an illness later diagnosed as "bronchitis."

Effect of Vaccination on Serologic Test for Syphilis. Since no information was available as to the effect of this vaccine and the resultant increase in A-I antibodies on a serologic test for syphilis,

Lt. Annis Wilkerson, WAC, kindly examined 2 or more sera from 59 of these subjects in the standard Kahn flocculation test. By 3 weeks, when the great majority of false positive reactions following smallpox vaccination are noted,¹³ none had shown any change.

Antigenicity of the Vaccine. When the 3 week post-vaccination sera were compared with those obtained at the time of vaccination, a 4-fold or greater rise in antibody was found for at least one of the component antigens in 83 (93%) of the study group. There were 55 individuals

for PR-8 3 weeks after vaccination, 65% for Weiss and 81% for Lee. It was also found that whereas only 9 individuals attained post-vaccination titers of A-I antibody of 1:256, or greater, for PR-8 and for Weiss, there were 31 with such levels for the Lee virus.

One of the most important considerations for any vaccine is the broadness of the antibody response it stimulates. Although the specificity of influenza viruses is less evident with human sera than with those from animals, it is well established that a satisfactory vaccine for clinical use

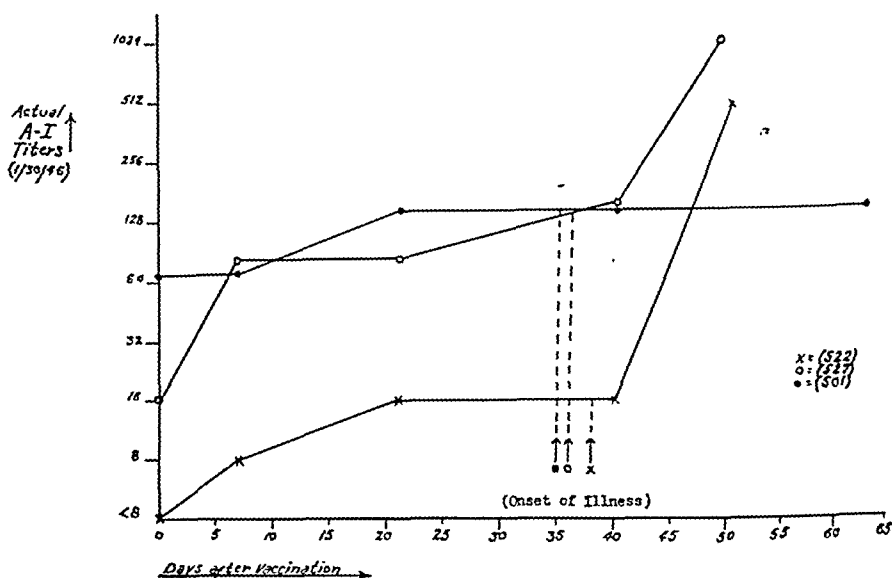


CHART 1.—Effect of vaccination and subsequent respiratory illness on A-I antibody for Type B (Lee) influenza.

(62%) who developed significant increases for both a Type A and the Type B strains. Eighteen (20%) showed such a rise for the Type B virus alone and 10 (11%) did so only for a Type A strain. There were just 6 (7%) in whom the vaccine failed to elicit any significant rise in antibody.

We have no data available as to the actual amount or distribution of specific influenza virus protein in the vaccine. However, if one accepts the resultant A-I titers as indications of antigenicity, interesting results are obtained. In Table 1 it is shown that 56% of the entire group had 4-fold or greater rises in A-I antibody

must contain both A and B strains. It is perhaps not so well recognized that there are definite antigenic differences among individual members within these types. However, it was this that prompted the inclusion of the Weiss Type A strain, which had been isolated in Michigan early in 1943,¹⁶ in the vaccine along with the well-established laboratory strains, PR-8 and Lee.

In order to examine the vaccine in respect to this broadness of antigenicity, we subjected the sera from our groups to A-I tests against 2 recently isolated influenza viruses. As a representative of

Type A the so-called Olson strain was obtained from Dr. M. D. Eaton who had isolated it from a patient in California during the fall of 1943.⁴ The Saha, Type B, strain was obtained from Lt. M. M. Sigel who had isolated it during an outbreak at Camp Atterbury, Indiana, in the spring of 1945.¹⁸

Type B. It shows that in general the pre-vaccination antibody for the 2 A strains which are in the vaccine (PR-8 and Weiss) and for the test strain (Olson) are quite similar. However, the antigenic stimulation for Olson is less striking than for either of the other 2 strains. There were 9 individuals who attained levels of

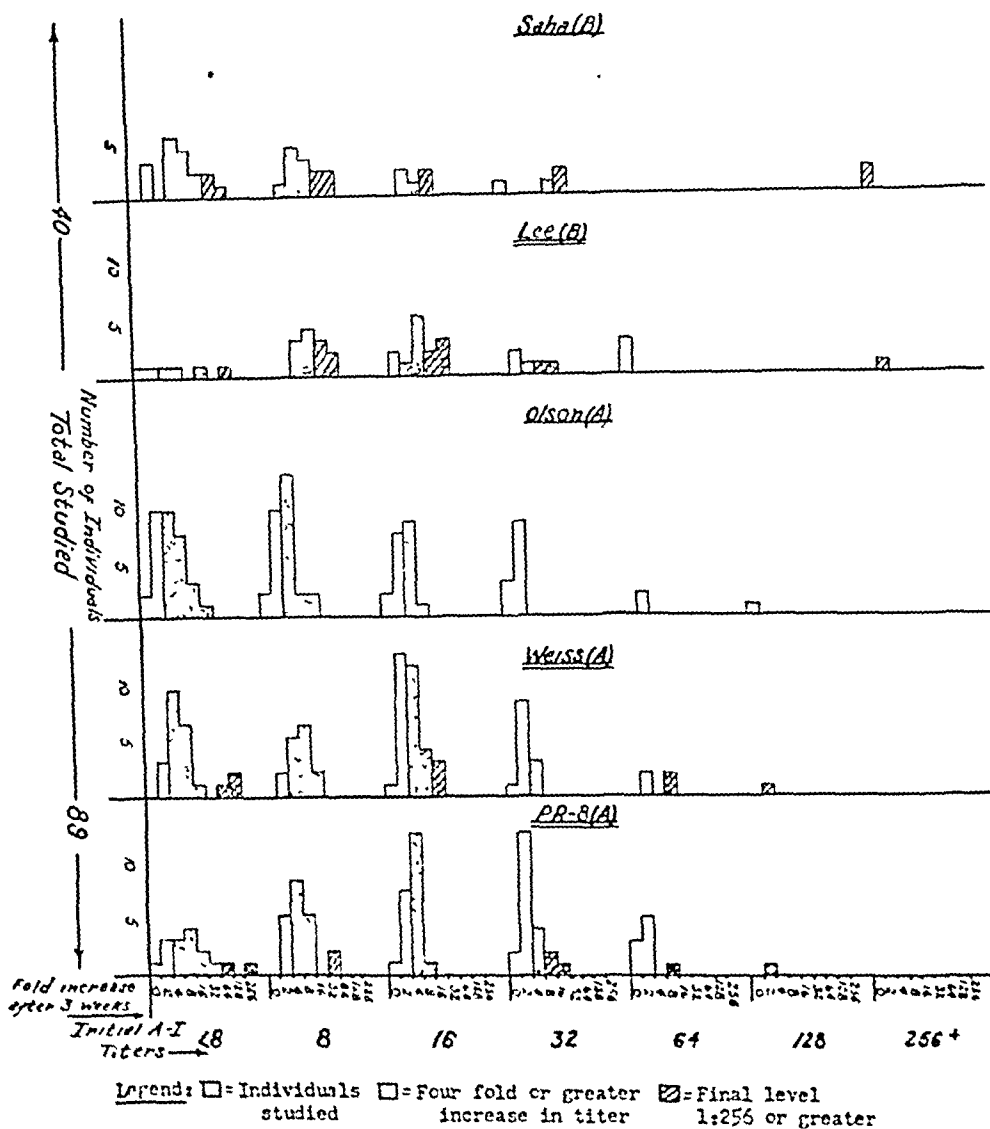


CHART 2.—Comparative antigenicity of components of vaccine.

In Chart 2 there is summarized for comparison the titers obtained at the time of vaccination and the antibody increases evoked by vaccination. Such comparative data was available for 89 individuals for the Type A strains and for 40 of the

1:256 or greater for PR-8 and for Weiss, but none who did so for Olson. In contrast both the pre- and post-vaccination levels for the Lee and the test strain (Saha) are quite similar. There were 14 individuals who attained post-vaccination

levels of 1:256 for Lee and 13 for Saha. It would seem that either the Saha strain is antigenically more nearly like the Lee than the PR-8 and Weiss are like the Olson, or that the antibody response evolved by the Lee virus is not only quantitatively greater, as has already been shown, but also is immunologically broader than either of the A strains in the vaccine.

Individual Variations in Response to Antigens. As has already been indicated, there were marked differences in the manner in which individuals responded to this vaccine. Some produce significant increases in antibody for all of the components, while others did so for only certain ones. There were even a few who did not apparently respond to the antigens administered but did demonstrate 4-fold, or greater, increase in antibody when tested against the Olson or Saha strains. In Table 2 there are listed a few representative types of response by individuals all of whom initially had low A-I titers. If the amount of circulating A-I antibody produced is proportional to an individual's protection against infection, this variability of immunologic response is a serious failing of the present vaccine.

Discussion. The all important question of whether this vaccine protects against an epidemic of influenza due to a Type B virus could not be answered because of the limitations imposed by the size and nature of the study group. However, with the aid of the A-I test it was possible to gather immunologic data and to attempt a correlation with clinical observations.

It is perhaps significant that the 2 proven instances of Type B influenza were in men who had failed to develop high levels of antibody for the B component of the vaccine. These unpredictable responses present a difficult problem.

Every comprehensive study of human antibody response to an immunizing agent has uncovered a proportion of individuals who develop little antibody and there were a few such non-reactors in our group. Effective immunization against influenza

is probably impossible for them.³ Our data also indicates that there are individuals who respond only to certain parts of the mosaic of antigens which make up an influenza virus so that they subsequently may have higher levels of antibody for related viruses than for those used as antigens. A similar phenomenon has been reported in enteric fever.² In some instances, especially early in the disease, the agglutination titer with the homologous *Salmonella* is much lower than with a related type. The effect of this kind of reaction on protection against future influenzal infections is not yet known. It awaits further study.

Summary. 1. Immunologic data were obtained with the aid of an agglutination-inhibition (A-I) test from 89 individuals who had been given a concentrated influenza virus vaccine containing 2 Type A (PR-8 and Weiss) strains and 1 Type B (Lee).

2. This information was analyzed in relation to clinical observations associated with the administration of the vaccine.

3. Almost two-thirds of the group complained of either local or systemic reactions shortly after vaccination. However, these symptoms did not seem to be the result of an antigen-antibody reaction involving A-I antibody since the distribution of pre-vaccination levels of antibody was in general similar among those who did not have such complaints as among those who did. Neither was it possible to associate an individual's immediate reaction to the vaccine with his subsequent production of antibodies.

4. Two serologically proven instances of Type B influenza infection occurred during the 5th week following vaccination. Both were in men who had not produced high levels of antibody for the B component of the vaccine.

5. A-I antibodies for the Type A viruses tended to appear earlier than for Type B. There were 17 individuals (20%) who reached their peak level for a Type A strain only at 1 week, but just 6 (7%) who did so for the Type B.

6. Administration of the vaccine and the resultant changes in A-I antibodies did not change the Kahn flocculation serologic test for syphilis reagin titer in the 59 individuals so studied.

7. By 3 weeks after vaccination, 83 (93 %) of the group had developed 4-fold, or greater, rises in antibody for at least 1 component of the vaccine. There were 56 % who did so for PR-8, 65 % for Weiss and 81 % for Lee. Although only 9 individuals attained titers of 1:256 for PR-8 and for Weiss, 31 did so for Lee.

8. The sera were also tested against recently isolated Type A and Type B viruses (Olson and Saha) in order to study the broadness of antibody response. The results indicated that either the Saha strain is antigenically more nearly like the

Lee than the PR-8 and Weiss are like the Olson, or that the antibody response evoked by the Lee virus is immunologically broader than that of the A strains in the vaccine.

9. Individual variations in serologic response to the vaccine were striking. Among those who responded there were some who did so to all of the components of the vaccine, some who did so only to certain ones, and a few who did not produce antibodies to the viruses in the vaccine as well as they did for the related Olson and Saha strains. The unpredictability of this phenomenon presents a serious problem if the level of circulating A-I antibody produced is indicative of the amount of protection against infection afforded by a vaccine.

The technical assistance of Sgt. Fred Trader, Miss Frances Condie and Mr. John Long was helpful in completing these studies. The charts were photographed by the U. S. A. Signal Corps.

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THE ELECTROCARDIOGRAPHIC EFFECTS OF PROSTIGMIN*

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TACHYCARDIA remains a difficult problem therapeutically and often interferes with the proper evaluation of the myocardial status in the interpretation of the electrocardiogram. Removal of the cause of the increased heart rate is to be recommended, but frequently, no cause is found. Recently, reports^{3,4,5} have appeared extolling prostigmin for all forms of tachycardia, including: simple or sinus; paroxysmal, ventricular or supraventricular, whether functional, organic or idiopathic. If this material is effective for the purposes recommended, it deserves wider use, not only as a therapeutic measure, but, as a diagnostic aid, since, electrocardiographically, a more accurate appraisal of the myocardium should be possible through slowing of the rate. The following report is based upon the study of 42 patients, appraising the effectiveness of this drug.

Material and Method. Patients selected for study were from private practice and from the cardiac clinic of The Chicago Medical School. The types of conditions present may be seen in Tables 1 and 2. The ages of the patients varied between 11 and 75 years, with a fairly even distribution between the ages of 20 and 70 years. There were 15 males and 27 females.

The patients were not informed of the intent of the study. They were conducted through the experiment as though it was the usual electrocardiographic technique employed. After a short rest period, the usual leads were taken. Then classical Lead 2 was retaken while 2 ampoules of 1:2000 prostigmin methylsulfate† solution (1 mg.) were administered subcutaneously. The time of the injection was marked upon

the film by a standardization. A short strip of film (Lead 2) was taken at 5 minute intervals at 5, 10, 15, 20, 25 and 30 minutes after the injection, the patient remaining in recumbency.

Results. The effect of prostigmin upon the rate of the heart measured electrocardiographically may be seen in Table 1. In this table the patients are classified according to the clinical diagnosis. It is noted that in no instance was the rate increased, whereas, in 26 (62%) the rate was slowed 20 or more beats per minute, and in 10 (24%) the rate was slowed between 10 and 20 beats per minute. The heart rate remained unchanged or was decreased less than 10 beats per minute in 6 (14%) of the patients.

The greatest effect was usually noted in the tracing taken 20 minutes after the injection. Occasionally, the most marked slowing was noted at 15 minutes, while some individuals showed the greatest slowing after a 25 minute interval. However, this variation was slight and for practical purposes, one tracing taken after 20 minutes should give the average major response obtainable. Usually, the rate increased slightly after 25 minutes, and this tendency became pronounced at the 30 minute interval, indicating the short duration of the effect of the drug.

When the effect of prostigmin upon the cardiac rate was measured according to the electrocardiographic classification (see Table 2), again it may be noted that in no instance was the rate increased, regardless of the mechanism present. Using the slowest rate obtainable (usually at the

* "Read by Title at the 18 Annual Meeting of the Central Society for Clinical Research, November 2-3, 1945"

† The material used was supplied by Hoffmann-LaRoche, Inc., Nutley, N. J.

20 minute period), 86% showed a slowing of 10 beats per minute or more. Frequently this was over 40 beats per minute. In 6 instances there was no effect upon the rate: namely, sinus mechanism in a patient with hyperthyroidism undergoing treatment with thiouracil; 2 instances of sinus tachycardia in patients with untreated hyperthyroidism; paroxysmal auricular

to a decrease in the rate. Examples are shown in Figure 1. In 2 individuals with sinus tachycardia the P waves became inverted during the height of the effect of the drug. This result was evanescent and frequently may have been missed. In 1 instance (hyperthyroidism, Fig. 1 *D*), the P wave was inverted only at the 20 minute interval; in the other example

TABLE 1.—EFFECT OF PROSTIGMIN UPON THE HEART RATE OF 42 PATIENTS CLASSIFIED ACCORDING TO CLINICAL DIAGNOSIS

Clinical diagnosis	No. patients	Rate slowed 20/min. or more	Rate slowed 10 to 20/min.	Rate slowed less than 10/min.	Rate increased
Neurocirculatory asthenia	14	8	6	0	0
Hyperthyroidism	6	3	0	3	0
Rheumatic heart disease	7	5	2	0	0
Hypertensive heart disease	6	4	1	1	0
Arteriosclerotic heart disease	1	1	0	0	0
Coronary artery disease	3	2	0	1	0
Miscellaneous:					
Allergy	1	0	0	1	0
Alcoholism	1	1	0	0	0
Abscess of leg	1	1	0	0	0
Thyroid extract poisoning	1	1	0	0	0
Idiopathic	1	0	1	0	0
Total number	42	26	10	6	0
Per cent	100	62	24	14	0

TABLE 2.—EFFECT OF PROSTIGMIN UPON THE HEART RATE OF 42 PATIENTS CLASSIFIED ACCORDING TO ELECTROCARDIOGRAPHIC DIAGNOSIS

Electrocardiographic diagnosis	No. patients	Rate slowed 20/min. or more	Rate slowed 10 to 20/min.	Rate slowed less than 10/min.	Rate increased
Sinus mechanism	4	2	1	1	0
Sinus arrhythmia	4	1	3	0	0
Sinus tachycardia	21	15	4	2	0
Paroxysmal auricular tachycardia	3	2	0	1	0
Paroxysmal ventricular tachycardia	1	0	0	1	0
Auricular fibrillation	5	4	1	0	0
Auricular flutter	2	2	0	0	0
Left bundle branch block	1	0	1	0	0
Auriculoventricular block	1	0	0	1	0
Total number	42	26	10	6	0
Per cent	100	62	24	14	0

tachycardia in a patient with hypertensive heart disease; paroxysmal ventricular tachycardia in a patient with a myocardial infarct; and, auriculoventricular heart block in a patient with allergy, who frequently complained of rapid heart action, but when the test was done the rate before the injection was only 70 beats per minute.

There were several changes noted in the form of the electrocardiogram in addition

(neurocirculatory asthenia, Fig. 1 *E*), the P wave inversion was noted only at the 15 minute interval.

The P-R interval was uninfluenced by the drug except in 1 instance of neurocirculatory asthenia in which the P-R was reduced from 0.18 to 0.16 second. In 1 case of rheumatic heart disease the P-R interval was increased from 0.16 to 0.18 second. In Figure 1 *B*, it is difficult to

measure the P-R interval before the drug was administered because of the fusion of the P and T waves. This becomes much more clearly defined after the injection; since there is remarkable constancy of this measurement throughout the series, there is no reason to assume much change in this example of first-degree heart block.

In 2 instances of sinus tachycardia the QRS complexes became heightened as the

rate decreased, although this effect was minor. The QRS complex was increased in amplitude when the mechanism changed from supranodal tachycardia to sinus tachycardia (Fig. 1 *H*) in a patient with an acute myocardial infarct. The paroxysm stopped 2 minutes after the injection of prostigmin when the patient vomited. It was impossible to state that the drug was responsible for arresting this

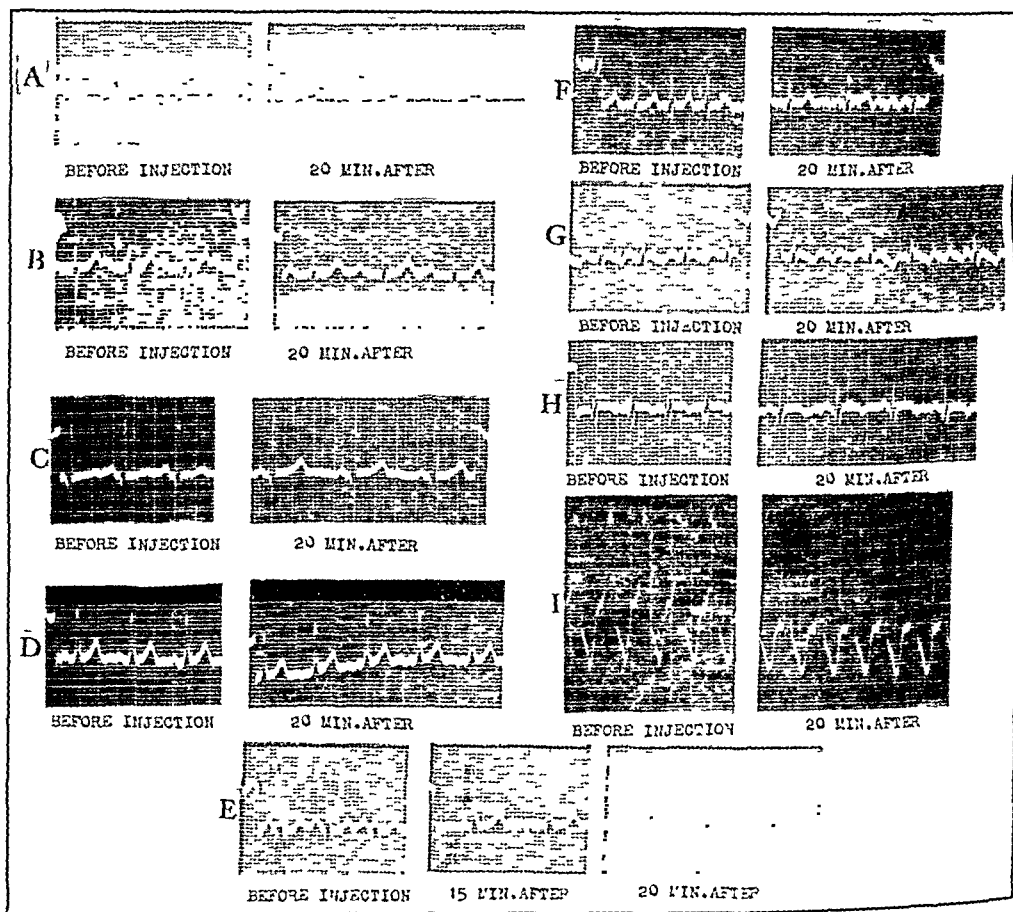


Fig. 1.—All tracings shown are classical Lead 2 before and 20 minutes after injection of 1 mg. of prostigmin methylsulfate. *A*, Note the marked slowing which resulted (125 beats to 74 beats per minute) in a case of thyroid extract poisoning.² *B*, Hypertensive heart disease: the slowing after injection permitted accurate appraisal of the prolonged auriculoventricular conduction time. *C*, Note increase in height of the T waves 20 minutes after injection. The rate was slowed. Clinical diagnosis: abscess of leg. *D*, Inversion of the P waves without slowing of the rate in a case of hyperthyroidism. *E*, Transitory P wave inversion at 15 minutes which has returned to normal at the 20 minute interval. The rate was decreased in this case of neurocirculatory asthenia. *F*, In a case of arteriosclerotic heart disease with auricular fibrillation, the ventricular rate was markedly slowed after the injection of prostigmin. *G*, In a case of hypertensive heart disease the previously regular 2:1 auricular flutter became irregular, increasing the block. *H*, The heart rate slowed abruptly 2 minutes after injection. The mechanism (supranodal tachycardia postmyocardial infarction) reverted to a sinus tachycardia and the amplitude of the QRS was increased. *I*, The rate during an attack of paroxysmal ventricular tachycardia (after a myocardial infarct) was uninfluenced by the injection of prostigmin.

attack, since a subsequent paroxysm was not aborted similarly.

The T waves increased in amplitude in 4 instances of sinus tachycardia. The clinical diagnoses were: abscess of leg (Fig. 1 C) hyperthyroidism, neurocirculatory asthenia, and rheumatic heart disease. The rate was unaffected in paroxysmal ventricular tachycardia but the T waves were more definite in form. This is apparent, although the difficulties of standardization may have been responsible for some of this effect.

The ventricular rate was slowed in 5 cases of auricular fibrillation (Fig. 1 F), and in 2 cases of auricular flutter (Fig. 1 G).

Discussion. The most pronounced effect of the injection of 1 mg. of prostigmin methylsulfate was upon the cardiac rate, which was slowed in 86% of the cases. While this slowing may have been as small as 10 beats per minute, yet a similar result could not be obtained with any other method: namely, carotid sinus pressure, rest, sedation, etc., when, and at the time this effect was desired, and according to electrocardiographic measurement.

Waldman and Moskowitz^{4,5} reported that the drug had no effect upon "constant tachycardia" associated with definite disease, but Bram¹ has refuted this. In our 6 cases of hyperthyroidism the sinus tachycardia was slowed in 3. Of the 3 cases unaffected, 1 had been treated with thiouracil and the tachycardia was no longer present. Of course, the effect was only temporary, tending in all instances to return to the earlier increased level after the 30 minute interval. This agrees with the findings of Waldman and Moskowitz.

Pelner³ reported the increased effectiveness of carotid sinus pressure in conjunction with prostigmin in restoring the normal rhythm in cases of paroxysmal tachycardia. No such effect was noted in our 3 instances. While the paroxysm stopped in 1, the result came so soon after the injection (2 minutes) that it would be impossible to ascribe the cessation of the attack to the drug. The rate was decreased in the other 2 cases, but the attack

was not stopped either with the drug alone or with the energetic application of carotid sinus pressure.

The effects of the drug orally were disappointing. Prostigmin bromide (15 mg.) tablets were administered. The rate was not slowed as dramatically and the deceleration achieved by the subcutaneous route could not be maintained by the use of the oral preparation. When a dosage large enough to produce notable slowing was used orally the unpleasant side-effects became so marked that only rarely could the drug be tolerated.⁴ It had been hoped that following an injection the oral preparation could be used with better therapeutic effect. This result was not achieved.

The side-effects encountered usually had passed off within a 2 hour period after an injection. The symptoms consisted of nausea, abdominal pains, twitching of the eyelids, tightness of the skeletal musculature of the jaws producing a speech difficulty and giddiness. These were more alarming in the group classified as neurocirculatory asthenia, but in no case severe enough to warrant the use of the antidote, atropine sulfate.

Most of the effects upon the form of the electrocardiogram are explained upon the basis that the rate is slowed by stimulation of the parasympathetics. This slowing permitted a more normal contour of the T wave to appear in 4 instances of sinus tachycardia. This effect probably also accounts for the occasionally noted increase in the QRS amplitude. No explanation is offered for the transitory inversion of the P waves.

For future work an electrocardiogram using classical bipolar and multiple unipolar leads will be compared before, and 20 minutes after the injection. The method followed in this series has established that the 20 minute period will permit the recording of the maximum change produced. When multiple leads are compared in this manner, clues to the myocardial state will undoubtedly become more apparent, justifying the procedure

as a test in selected cases. This, in effect, constitutes a reversal of the well-known electrocardiographic effect of proving nutritional insufficiency by increasing the heart rate by lowered oxygen tension or exercise tests.

Summary and Conclusions. 1. The effects of 1 mg. prostigmin methylsulfate upon the electrocardiograms of 42 patients were observed.

2. The cardiac rate was slowed in 86% of the patients studied, including both organic and functional conditions.

3. Besides a decreased rate, other effects were noted, including transient inversion of the P wave, increase in amplitude of the QRS, increase in the amplitude of the T waves, separation of a fused T and P wave in auriculoventricular block, slowing of the ventricular rate in auricular fibrillation and auricular flutter.

4. The effect of the oral preparation of prostigmin bromide was disappointing when used therapeutically.

5. The procedure recommended is to compare complete electrocardiograms before and 20 minutes after the injection.

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THE TOXIC EFFECTS OF PROLONGED INGESTION OF DDT* ON DOGS WITH SPECIAL REFERENCE TO LESIONS IN THE BRAIN

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TREMOR followed by incoördination, muscular twitching, flaccidity, and other manifestations of impairment of the central nervous system have been observed in a variety of animals given DDT (Cameron and Burgess,² Draize, Nelson and Calvery,⁴ Lillie and Smith,⁶ Neal and associates,^{7,8} Orr and Mott,¹⁰ Smith and Stohlgman,¹² Nelson and Calvery¹⁴). Similar symptoms, lasting for months, developed in a man subsequent to inunction of an acetone solution of DDT (Wigglesworth¹³). The literature contains 2 reports on the clinical manifestations of DDT intoxication in dogs. Nelson and associates⁹ observed tremor in 2 dogs given a total of 2.8 gm./kg. of DDT in corn oil by stomach tube for 1 month, and in a third which received 10.5 gm./kg. of DDT in the same manner for 7 months. Draize, Nelson and Calvery⁴ found that animals displayed no ill effects throughout 9 months during which they received by inunction a total of 0.3 to 1.2 gm./kg. of a 30% solution of DDT in dimethyl phthalate. The clinical and certain of the pharmacologic aspects of DDT intoxication in 28 dogs, from 4 of which many of the data for our study have also been gathered, have been reported by Bing, McNamara and Hopkins.¹

Studies of the central nervous systems of animals after administration of DDT have yielded little of note. Cameron and Burgess² gave a total of 0.8 gm./kg. of DDT to rabbits over a period of 16 days, and 1.5 gm./kg. to rats for 30 days (the DDT in each instance being suspended in liquid paraffin and tragacanth and admin-

istered by stomach tube). Degenerative changes took place in anterior horn cells of the spinal cord but not in the cerebrum, cerebellum or brain stem. Lillie and Smith⁶ had much the same experience with cats, rabbits and rats. Complete lack of change in the central nervous system of a variety of animals, including the dog, was reported also by Nelson and associates.⁹

Of the pathologic changes in the thoracic and abdominal viscera, the most notable have been in the liver. These consisted either of (1) centrilobular cytoplasmic oxyphilia or hyaline degeneration or (2) centrilobular and midzonal fatty degeneration progressing to focal or zonal necrosis or to atrophy. The only change observed in the kidney was that of fatty degeneration of tubular epithelium, usually mild to moderate in degree. It is apparent from the various reports in the literature that visceral lesions occurred only after the administration of relatively large amounts of DDT.

Methods. The dogs given DDT and those used as controls for our study were chosen at random from the kennels at Edgewood Arsenal. All were mongrels, most of them females; they were from 1 to 4 years old, and weighed from 6.1 to 18 kg. All were on an ample diet supplemented by vitamins and were in excellent health before the experiment was started.

Eight dogs comprised the experimental series. They were given a solution of 10% DDT in peanut oil by stomach tube in doses ranging from 150 to 350 mg. kg. The prepa-

* 2, 2-bis (p-chlorophenyl)-1,1,1-trichloroethane.

ration was given daily except when the systemic reaction brought about by previous dose was excessive; sometimes several days elapsed before use of the preparation was resumed. When the animals were too ill to eat, no attempt was made to force-feed them. There were 9 controls; 5 untreated, 2 which received peanut oil by stomach tube for 90 days in an amount equivalent to that used as a solvent for the DDT given in experimental animals (3 cc./kg. daily), and 2 which were given peanut oil and subsequently fasted until they lost 20 and 31% of their body weight respectively.

Four of the animals given DDT died, and the remainder, as well as the controls, were sacrificed by means of intracardiac injections of 10 cc. of nembutal. All of the autopsies were done promptly. The brain, spinal cord and samples of the sciatic and vagal nerves were fixed in 10% formalin, and blocks of the thoracic and abdominal viscera in Zenker's solution. The eyes of 2 of the experimental animals and of 1 of the controls also were fixed in 10% formalin. After fixation, the cerebrum was sectioned in the coronal plane at 7 representative levels, the brain stem at 3, the cerebellum at 3, and the spinal cord at 4. The material was embedded in paraffin. The sections of the brain and spinal cord were stained routinely with cresyl violet and those of the viscera and the eyes with hematoxylin and eosin. In certain instances, frozen sections from the central and peripheral nervous systems were stained for fat by scarlet red, for myelin by the Spielmeier method, and for axis cylinders by the Bodian method.

Clinical Observations.* In general, the symptomatology varied in accordance with the amount of the daily dose of DDT. Large doses given for a few days usually induced more severe symptoms than smaller doses over a prolonged time. Tremor constituted the earliest manifestation of DDT intoxication. Initially it was fine, generally starting in muscles of the eyelids and face and spreading with variable sequence and severity to all muscles. The tremor occurred spontaneously when the animals were at rest, but was accentuated by excitement or

movements, being most intense on completion of a movement. On daily doses ranging from 150 to 200 mg./kg. the tremor usually did not develop until several days had elapsed, but sometimes became apparent after the first dose; it began 4 to 6 hours after DDT had been given, generally lasted for several hours, and remained reversible after prolonged administration. Three examples in this category are listed in Table 1 (Nos. 133115, 159669 and 159668).

When, however, the daily dose of DDT ranged from 180 to 250 mg./kg., or higher, the course was one of steady decline. Anorexia, listlessness and weakness set in, weight loss occurred in 4 (Table 1), and eventually death occurred. Tremor developed after the first administration of DDT and except for 1 instance (No. 159-673), became more coarse and widespread than in animals given a lower daily dose. Moreover, the animals (Nos. 147733, 147732, 159672 and 135777) became ataxic and developed hypermetria, which, together with the tremor, ultimately became irreversible. On standing, such animals were unsteady, and their limbs usually were hyperextended and abducted. They tended to stagger when walking, lifted their legs unduly high with each step, and often were unable to arrest locomotion, with the result that they bumped into clearly visible objects. After a time the stretch reflexes of the limbs became overactive; thus, when the head was thrust backward the tone of the forelimbs was considerably augmented, and when the head was tilted to the right or the left the hindlimb of the corresponding side underwent extreme extension. Also the placing reactions were strongly positive, i. e., when the animals were held in the air with eyes shielded to occlude vision, the slightest contact of the anterior aspect of a paw with the edge of a table resulted in an exaggerated flexion of the limb, followed by maximal extension. No disturbance of sensibility was detected, nor were

* The clinical observations herein described were made jointly by Bing, McNamara and Hopkin¹² and ourselves.

TABLE 1.—THE DOSEAGE OF DDT AND THE APPROXIMATE DEGREE OF DEGENERATION OF THE CEREBELLAR NUCLEI
Cell counts of the cerebellar nuclei in 30 low power fields
(X 100) and the degree of degeneration

Alp. dog No.	Average of daily dose (mg./kg.)	No. doses	Total dose (gm./kg.)	Period over which DDT was given (days)	Period of survival (days)	Severity of neurologic symptoms*	Weight loss (%)	Died or sacrificed	Total cells	Normal cells	"Abnor- mal" cells†	"Abnor- mal" cells† %	Degenera- tion (%)
147733	250-300	40	7 46	103	110	+++	27	S	1901	973	928	48 8	36 9
147732	200-250	42	7 16	98	99	+++	0	D	1875	718	1157	61 7	49 8
159672	300-350	21	5 10	28	28	+++	0	D	2130	1440	690	32 4	20 5
155777	150-250	14	2 16	17	18	+++	18	S	2508	1926	582	23 2	11 3
133115	150-200	21	3 74	24	25	++	0	S	2415	1918	467	19 3	7 4
159669	150-200	9	1 70	13	13	++	14	D	1925	1655	270	14 0	0
159673	300-350	21	5 95	28	28	+	44	D	2055	2378	277	10 4	0
159668	150-200	10	1 90	13	13	+	0	S	2605	2314	291	11 2	0
<i>Untreated Controls</i>													
153842	0	S	2012	1744	208	14 6	0
153843	0	S	2253	1985	268	11 9	0
153844	0	S	2318	1974	244	11 0	0
153845	0	S	2501	2190	311	12 4	0
153846	0	S	2308	2082	226	9 8	0
<i>Peanut Oil—Treated Controls</i>													
159671	90	...	16	S	2080	2366	324	12 1	0
159670	90	.	0	S	2132	1815	315	14 8	0
<i>Peanut Oil—Treated and Fasted Controls</i>													
162957	90	..	31	S	2690	2501	189	7 0	0
167958	90	..	20	S	2520	2274	246	8 4	0

* Irreversible symptoms are indicated by 3+ and 4+ and 1+, reversible ones by 1+ and 2+.
† A sharp line of distinction between faded and actually degenerated cells could not be made—hence the grouping together of all cells not having a normal appearance.

there defects of vision or hearing. None of the animals had convulsive seizures.

The 2 dogs given peanut oil by stomach tube over a period of 90 days had no untoward symptoms, and appeared to be as healthy at the termination of the experiment as at the beginning; 1 lost 16% of its body weight, while the other gained an equivalent amount. Lack of symptoms was also apparent in the other 2 controls so long as peanut oil was given; when, in addition, they were fasted they lost weight but otherwise suffered no visible untoward effects.

Gross inspection of the brain and leptomeninges of the dogs given DDT revealed moderate congestion and occasional petechial hemorrhages. The ventricles appeared to be of normal size. Microscopic examination revealed nothing of significance in the cerebrum, brain stem, peripheral nerves, or eyes. At all levels of the spinal cord, there was a normal complement of anterior horn cells but some of them displayed slight to moderate chromatolysis.

The cerebellum showed varying degrees of damage. In animals in which the level

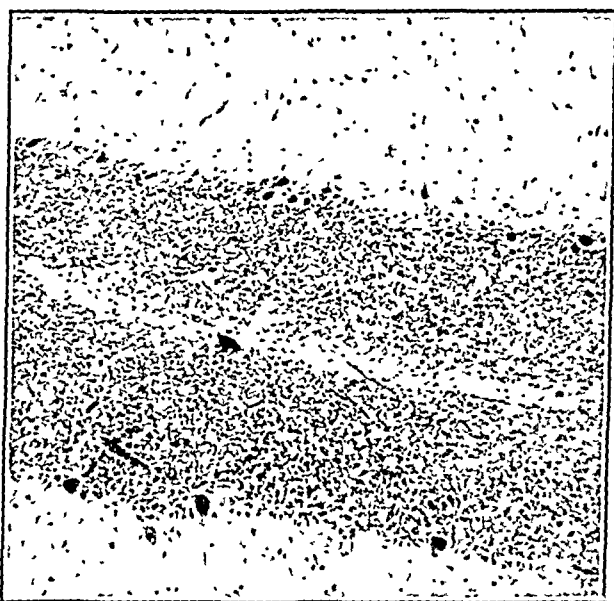


FIG. 1.—(Dog 147732; total dose of DDT, 7.16 gm./kg.). Many of the cells of the cerebellum are shrunken and otherwise distorted, and a few have disappeared. The molecular, Bergmann, and granular layers show nothing of significance. (Cresyl violet, $\times 145$.) (AIP neg. 90295.)

Pathologic Anatomy. Examination of the thoracic and abdominal viscera of the animals receiving DDT revealed constant lesions only in the liver. The most characteristic of these consisted of sinusoidal engorgement in association with fatty degeneration of hepatic cells, usually centrilobular in distribution but sometimes midzonal as well. Hemosiderosis of Küpf-fer cells and centrilobular atrophy were observed in some of the cases of longer standing. No significant changes were found in the other tissues.

of dosage of DDT ranged from 150 to 200 mg./kg. a moderate number of Purkinje cells exhibited swollen and hyperchromatic cytoplasm and pyknotic nuclei to a degree not observed in the controls. The dentate and roof nuclei, however, were not convincingly abnormal except in 1 instance (No. 133115) in which mild degeneration was apparent (Table 1). Moderate to severe degenerative change in the cerebellum was observed in all animals given 180 to 350 mg./kg. of DDT, except in 1 instance (No. 159673). In 2

of the animals receiving the largest doses over the greatest length of time (Nos. 147733 and 147732) many of the Purkinje cells were pyknotic or had been converted into "ghosts," and a few had disappeared (Fig. 1). There was no hyperplasia of cells of the Bergmann and molecular layers, nor were changes detected in the granular layer. The dentate and roof nuclei were severely affected. As com-

An effort was made to determine quantitatively the degree of degenerative change in the dentate and roof nuclei. This was done by counting the number of normal and abnormal cells in 3 low power fields ($100\times$ magnification) of the 10 serial sections, making a total of 30 fields examined; the fields chosen were those in the mid-portion of the dentate nucleus of both sides and the fastigial nucleus of one

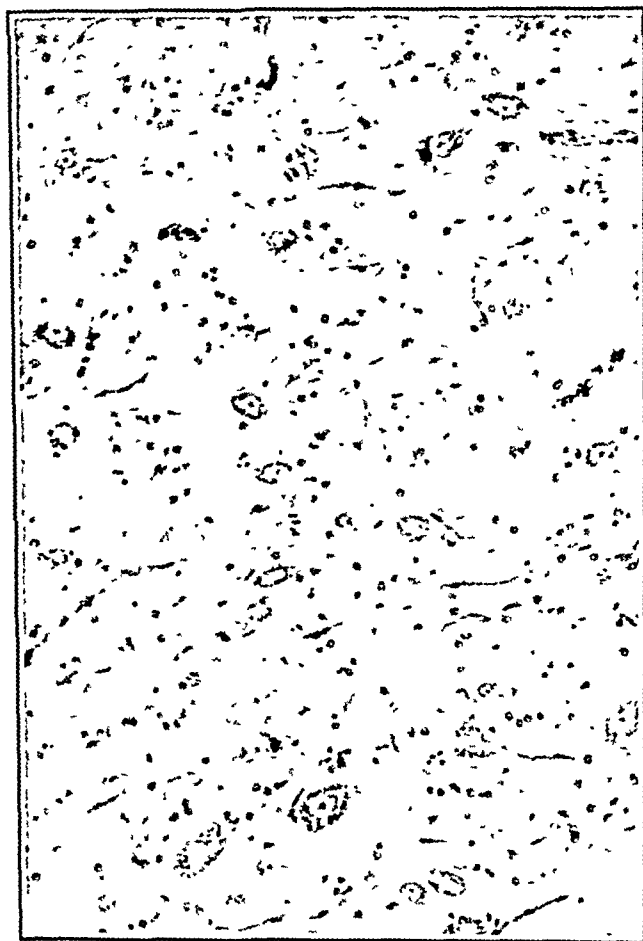


FIG. 2.—(Dog 153846; untreated control.) The ganglion cells in this field of the dentate nucleus are numerous and virtually all of them have the features of normal cells. (Cresyl violet, $\times 230$.) (AIP neg. 92211.)

pared to a section from a control (Fig. 2), the nerve cells were somewhat fewer in number, and many of those remaining displayed chromatolysis and vacuolization of the cytoplasm, and karyorrhexis or complete loss of nuclei; some cells were completely amorphous (Figs. 3 and 4). There was no generalized gliosis but the number of satellite glia was sometimes increased (Fig. 3).

The small aggregation of cells corresponding to the emboliform nucleus of man was also examined. Care was taken to cut all sections at 6 microns. At a later date the counting was repeated, and the average of the 2 counts taken as the final figure. Cells exhibiting mild chromatolysis or slight blurring of nuclear detail were included among the normal forms; those so hazy that cellular detail could not

be discerned and those obviously degenerated were listed as abnormal. The results, which are to be regarded as only approximate, are shown in Table 1. Abnormal forms in the untreated controls varied from 9.8 to 14.6%, and averaged 11.9%. (The abnormalities were ascribed to postmortem change or to artefact.) Approximately the same degree of altera-

figures on the over-all degeneration of this group, which varied from 7.4 to 49.8%, were obtained by subtracting the average % "abnormal cells" in the 5 untreated controls (11.9 %) from the % "abnormal cells" found in each of the dogs treated with DDT. Statistically evaluated, the index of nuclear degeneration in the first 6 animals listed in Table 1 were as follows:

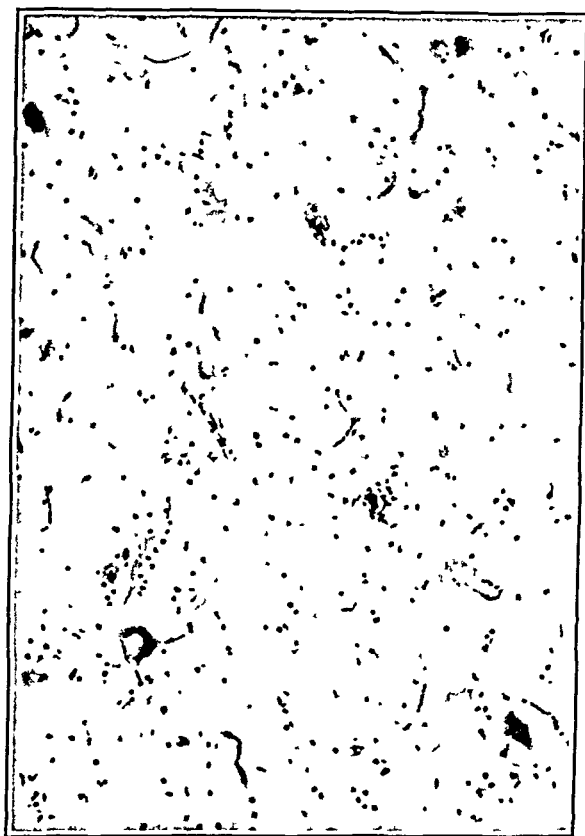


FIG. 3.—(Dog 147732; total dose of DDT, 7.16 gm./kg.) This field of the dentate nucleus contains 6 relatively normal cells and numerous faded or obviously degenerated forms. As compared to the control (Fig. 2), the actual number of cells is not greatly reduced. Satellites in the vicinity of 1 of the distorted cells are increased in number. (Cresyl violet, $\times 250$.) (AIP neg. 92266).

tion was observed in the controls receiving peanut oil and in the animal which had been fasted. In 4 of the animals in which the daily dose of DDT ranged from 180 to 350 mg./kg. (Nos. 147733, 147732, 159672 and 135777), and 1 in which the daily dose range was 150 to 200 mg./kg., a moderate to marked increase in the number of abnormal cells was found. The

20.7, 27.9, 11.5, 6.3 4.2, 1.2. The amount of cell destruction in the animals given DDT was not significantly large, but what there was, could be correlated with the total doses given, the value of the correlation coefficient being 0.76.

Discussion. From the clinical standpoint it seemed apparent that the severity of the symptoms depended more on the

amount of daily dose of DDT than on the total dose. Thus, Dog 135777 received a daily dose of from 180 to 250 mg./kg. DDT, amounting altogether to 2.47 gm./kg., and developed irreversible symptoms, whereas Dog 133115, on a smaller daily dose but a greater total dose was not affected clinically to the same degree. That tolerance to DDT varies is indicated

by the observation of degenerative change in the cerebellum. The data on the entire series would seem to indicate that damage to cells of the cerebellum is slowly progressive and probably irreparable.

The lack of changes in other portions of the central nervous system points to the cerebellum as the essential site of action of DDT. This view is supported

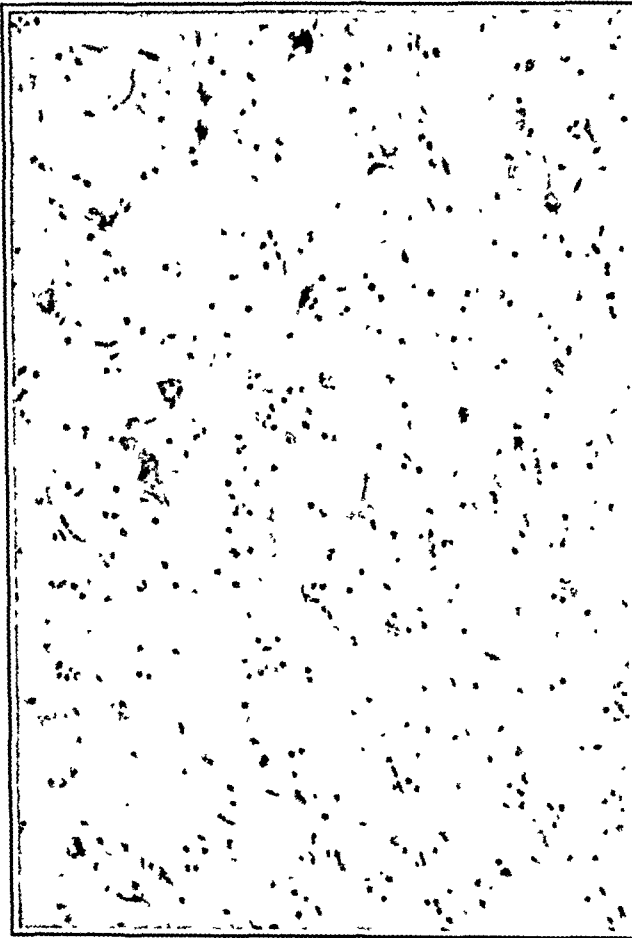


FIG. 4.—(Dog 147733; total dose of DDT, 7.46 gm./kg.) This also is a field of the dentate nucleus showing a preponderance of shriveled and otherwise distorted cell forms. (Cresyl violet, $\times 250$.) (AIP neg. 92261.)

by the failure of irreversible symptoms to develop in Dog 159673 despite high daily and total doses; judging by the loss of weight, this animal exhibited the most severe systemic reaction of the group. Two animals (Nos. 147733 and 147732) receiving large daily doses of DDT over the longest period had the most striking neurologic symptoms and the greatest de-

gree of degenerative change in the cerebellum. The data on the entire series would seem to indicate that damage to cells of the cerebellum is slowly progressive and probably irreparable. The lack of changes in other portions of the central nervous system points to the cerebellum as the essential site of action of DDT. This view is supported

* Personal communication to the authors.

tration of DDT. In 3 cats the spinal cord was sectioned at levels between the seventh thoracic and second lumbar segments. Eight to 20 days later each received by stomach tube 300 mg./kg. of DDT in peanut oil. Within 3 to 6 hours tremors developed in the muscles innervated above the transections, and potentials taken from these muscles showed a significant increase in action currents. The muscles below the transections failed to exhibit tremor, however, and no action potentials could be recorded from them. In 3 additional cats in which neurologic symptoms followed the administration of 300 mg./kg. of DDT, the brain stem was transected at the lower level of the mid-brain; 10 to 20 minutes later the symptoms reappeared. The conclusion drawn was that in *acute* experiments on the cat DDT exerts its effect on the portion of the central nervous system below the mid-brain and above the spinal cord, and that it apparently does not act on the spinal cord, the myoneural junctions, or the muscles.

Further evidence that the cerebellum may be the essential site of action of DDT has been gained from the electroencephalographic studies of Crescitelli and Gilman³ on cats and monkeys. In animals in which tremor induced by DDT was abolished by curare and in which a central depressant, such as sodium pentobarbital, then was given, the subsequent administration of DDT intravenously led to electroencephalographic changes in both the cerebral cortex and cerebellum. The main effect on the motor cortex consisted of a change from the normal pattern of irregular bursts of waves at a frequency of 8 to 12 cycles per second to one of persistent rhythm with almost continuous and regular discharges at the same frequency, whereas a progressive increase in the amplitude of activity took place in the cerebellum, attaining within 1 to 2 hours after the injection of DDT a constant peak voltage with slight increase in the wave frequency. The tentative conclusion reached was that DDT acts primarily on the cerebellum, and that as a result the discharges are

transmitted *via* efferent paths to the cerebral cortex, modifying its activity. When the use of a central depressant was avoided and the curarized animal anesthetized lightly with ether, periodic electrical disturbances of a convulsive character occurred simultaneously in the cerebral cortex and cerebellum, and continued for a number of hours. The disturbances were always heralded by fast spike-like waves which were localized to the premotor cortex of the cerebrum on the one hand, and from the pyramis vermis and lobulus simplex of the cerebellum on the other. These spike-like waves occurred synchronously from the 2 structures, but with opposite polarity, *i. e.*, they were of positive polarity in the cerebral tracings, and of negative polarity in the cerebellar tracings. For the present, these findings are still open to some difference of interpretation as to the primary source of the waves. The possibility that the cerebral and cerebellar responses to DDT may be due to the discharge of afferent impulses by way of the spinal cord appears to be excluded by the finding that the electroencephalographic changes produced by DDT were not altered when the spinal cord was sectioned at the atlantoöccipital level.

The presence of slight to moderate liver damage in most of the animals is in accord with the findings of Bing, McNamara and Hopkins¹ that liver function is but little impaired, except terminally, even when relatively large amounts of DDT are administered. The sparing of the kidneys also concurs with their studies on kidney function.

Conclusions. From clinical observations as well as physiologic and electroencephalographic data, it appears that the cerebellum is the chief portion of the nervous system on which DDT acts. The same would seem to hold pathologically, inasmuch as degenerative changes in DDT-intoxicated dogs were restricted to the cerebellum, especially the dentate and roof nuclei. The degenerative changes in the

cerebellum were regarded as slowly progressive, since they were found only when relatively large doses were given over prolonged periods of time.

The systemic symptoms which occurred

in some of the animals were not open to pathologic interpretation, since nothing of significance aside from slight to moderate degenerative change in the liver was observed.

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LATE NEURONITIS FOLLOWING PROVED AND SUSPECTED CUTANEOUS, FAUCIAL AND WOUND DIPHThERIA

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THE purpose of this report is to describe the clinical course of 20 cases† of late polyneuritis following proved or suspected cutaneous and pharyngeal diphtheria. This syndrome is to be differentiated from the early diphtheritic neuritis occurring during, and within the first month after, the disease.

As evidenced by certain personal communications from Liebow,¹² Livingood,¹³ and others, and by publications of Norris *et al.*,¹⁶ Williams,²⁴ and the War Department,²³ as well as the histories of our own overseas patients, there has been considerable diphtheria in the Southwest Pacific theater of military operations in 1942, 1943 and 1944 and most probably more in western Europe and the Continental United States than in previous years. It has been suggested that the infection may have existed in the Pacific theater in the native population or been introduced by the Japanese invaders of the Solomon Islands, the North New Guinea area, Saipan and the Philippine Islands. Both Schick negative and Schick positive men acquired the infections and the Schick test in the milder cases occasionally remained positive after cure. The disease may occur as ulcerative skin lesions, typical or subclinical faucial infection, or both skin and pharyngeal involvement. Either the skin or pharynx may be the primary source of infection which then may spread to other regions.

The occurrence of cases of typical Guillain-Barré syndrome following cutaneous diphtheria was noted in 1918 by Walshe²² during the first World War. Although

Wilson,²⁵ Grinker,⁸ Ederle⁶ and others recognized the frequency of secondary post-diphtheritic polyneuritis, there has been a paucity of reports indicating the frequency of association of this syndrome following diphtheria until statements of recent military clinical experiences. The War Department *Bulletin*²³ comments on the occurrence of peripheral neuritis in a fifth of all diphtheritic dermatitis cases.

It is stated that the neuritis occurs from 2 to 4 months after the onset of the infection and of the early cranial nerve palsies. It is associated with elevated spinal fluid protein and generally is characterized by lost reflexes, motor weakness, paresthesias and hypesthesias of the extremities, especially the lower legs and feet. Certain reports fail to separate these cases from the ordinary early diphtheritic peripheral neuritis which occurs within the 1st month after infection. Whereas no fatalities have been reported, exclusive of 1 case, probably an example of early diphtheritic neuritis, as reported by Norris *et al.*,¹⁶ many cases observed at Baxter General Hospital and in other continental and overseas hospitals have been very severe and have resulted, although infrequently, in significant motor or sensory residual disturbances. Some of these patients are so completely incapacitated at the height of their illness that they cannot lift their heads off the pillow or feed themselves.

The characteristic clinical picture of Guillain-Barré syndrome or infectious polyneuritis, or polyneuronitis, resembles these postdiphtheritic states. The Guillain-Barré syndrome may be summarized

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† An additional case of neuronitis following prolonged faucial diphtheria has not been studied for a long enough period to warrant inclusion in the series.

as follows: the onset generally follows 5 days to 6 weeks after an upper respiratory or other infection; the initial symptoms are largely sensory, including pain in the extremities. This is followed closely by a progressive, bilateral symmetrical quadriplegia (predominantly proximal) which overshadows the sensory signs. Cranial nerves are generally involved, often the seventh nerve. The cerebrospinal fluid typically shows a high protein content without increased number of cells. The mortality is reported from 14 to 42%. The convalescence is slow but usually complete—moving rapidly once started, and the duration generally varies from 14 to 120 days. Drug therapy, including thiamine chloride, is currently ineffective.

Although there has been considerable disagreement in the application of the terms neuronitis and neuritis to this syndrome, the term neuronitis is herein used since it implies involvement of the nerve cells, axones and dendrites of the brain, spinal cord, spinal, cranial and autonomic ganglia and cranial and peripheral nerves. From pathologic studies on autopsy cases of Guillain-Barré syndrome (Roseman and Aring,¹⁸ Lowenberg and Foster,¹⁴ and others^{3,4,5,9}) this widespread involvement has been demonstrated.

These pathologic findings may be briefly summarized as: 1. Shrinkage, vacuolization and destruction of nerve cells throughout the brain, spinal cord and ganglia, rarely extensive and occasionally associated with perivascular hemorrhage.

2. Axonal degeneration in the brain, spinal cord, especially the posterior columns, and cranial and peripheral nerves as evidenced by (a) swelling, corkscrewing, beading and fragmentation of the axis cylinders; (b) swelling and fragmentation of the myelin sheaths; (c) proliferation of the Schwann cells; and (d) occasional phagocyte and lymphocyte invasions—chiefly present in the peripheral nerves.

3. The spinal cord involvement was greatest in the cervical and upper thoracic and then in the lower lumbar regions.

The involvement was heavy in the vagus nuclei and the peripheral nerves.

In addition to nervous system lesions, visceral pathologic changes have been reported, namely: 1. Fatty infiltration, mononuclear and polymorphonuclear cellular accumulations and focal degeneration of the liver.

2. Adrenal cortical cell degeneration.

3. Interstitial cellular infiltration of the kidneys without nephron damage.

4. Necrosis of muscle fibers and mononuclear and polymorphonuclear cellular infiltration of the heart. McIntyre¹⁵ recorded electrocardiographic changes in a patient with Guillain-Barré syndrome, similar to those found in diphtheria, namely A-V conduction delay and S-T and T wave abnormalities.

An outline of the 20 cases is presented in Table 1. The patients varied in age from 20 to 35 years and there was only 1 female, Case 16, in the group. Three acquired pharyngeal diphtheria in the European theater of operations; 1 pharyngeal case was contracted in Spokane; 1 empyema with suspected diphtheria in Alaska; and the remaining 15 acquired pharyngeal or cutaneous diphtheria infections in the Southwest Pacific theater of operations. There were 62 cases of healed or active cutaneous diphtheria admitted into the hospital during the period from December 1 to February 15, of which 11 developed polyneuritis. All of these 11 cases are included in the series of the 20 cases outlined in Table 1. Ten neuronitis cases accompanied or followed clinical cutaneous diphtheria showing characteristic anesthetic, punched-out lesions and partially to completely anesthetic, sharply defined scars.

One patient had exfoliative dermatitis with irregular areas of local infection. Two patients had both clinical diphtheria and pharyngeal diphtheria. Five patients had clinical pharyngeal diphtheria, although virulent *C. diphtheriae* could not be cultured from 1 patient. Two patients had neuronitis accompanying resistant empyema with avirulent diphtheroids in

TABLE 1.—SUMMARY OF 20 CASES

Case No. Initials Age Theatre Severity	Clinical		Initial symptoms	Cranial nerves involved	Maximum involvement		
	Skin ulcers	Phar. dipl.			Clinical	Flaccid react. degen.	Reflexes
1. L D R. 35 Bial. ++++	+	0	Numb hands and feet	V, VII	3C-2T, 2L-3S, 60d-bed, arms, legs, trunk	Ptl +++	Abs Bt Tr AJ, KJ
2. A G 24 New Guinea ++++	+	+	Tingling, numb hands and feet, slapping gait	(Early III, IX, X, XI) III, V, VI, VII	3C-2T, 2L-3S, 48d-bed, arms, trunk, legs	Ptl +++	Abs Bt Tr AJ, KJ
3. J F S 25 New Guinea ++	+	0	Can't lift R arm, numb and tingling hands and feet, ache in arms legs	0	3C-1T, 3L-1S, can't comb hair, climb stairs, numb feet +	Ptl + R shoulder, L peron	Abs Bt Tr AJ, KJ
4. C F B 29 New Guinea ++++	Exfol Derm	0	Numb and tingling feet, stumbling gait	0	L 5L-1S, R 3L-1S, bed, arms and legs	Ptl +	Abs Bt Tr AJ, KJ
5. C A S 35 New Guinea +	+	0	Numb fingertips	V?	Fingertips and 3L-2S, R and L toe-drop, weak arms, hypesthesia, hands and feet	0	Abs Bt Tr AJ, KJ
6. H A O 24 New Guinea ++++	+	0	Tingling and numb fingertips, toes and soles	0	4C-3T, 3L-1S, bed-trunk, arms, legs and feet	Ptl +++ Trunk, arms and legs	Abs Bt Tr AJ, KJ
7. J McC 27 Alaska ++++	0	0 ? wound +	Numb face, arms and lower legs	V, VI, VII, VIII, X, XI, XII	5C-1T, 10T-1S, bed-ridden	Ptl gen Arms, legs, trunk and shoulders	Abs Bt Tr AJ AJ AJ d and Crem
8. R H W 20 Leyte +	+	+	Numb feet	0	7C-ST, 3L-1S, can't type or climb stairs	Ptl + Arms, legs	KJ =, AJ At
9. K A N 25 Leyte +	+	0	Numb and tingling fingertips, R ankle L toes	0	4C-3T, 3L-1S, toe-drop, can't climb	Ptl = to ++ gen	KJ =, Bt Tr AJ Als
10. D H F 29 New Guinea, Leyte +	+	0	Numb feet	III (accom)	4L-1S, toe-drop, can't climb	0	KJ =, AJ Als
11. F N B 29 Italy +	0	+	Numb fingers and toes	(X early) 0	Weak grip L and climbing	0	AJ Als
12. H H S 23 Leyte +	+	0	L toe-drop, numb L foot and ankle	0	Fell on walking, can't climb or hold arms up, toe-drop	Ptl + L leg	KJ, AJ, Abd Als
13. H G H 27 Leyte ++	+	0	Weak legs with climbing	0	7C-ST, 4L-1S, numb (stumbled), can't climb, weak grip	0	Normal
14. J W 33 Leyte =	+	0	Weakness and aching of lower legs	0	Numb fingers and R foot, loss vibr sense R foot, sl weak legs	0 0	Normal Normal
15. V T R 23 Belgium ++	0	+	Numbness, tingling of feet and fingers	(III, V, X, early) 0	Weak legs, slapping gait, can't hold fork or pen and L shoulder weak	Ptl +++ Arms, legs	L Bt Tr At L, KJ =, AJ Als
16. L H. 28 Spokane ++	0	+	Numb fingers and stumbling gait	(III, V, early) 0	Numb lower arms, hands, low legs, feet, can't walk or button clothes	Ptl Arms, legs	Abs Bt Tr KJ, AJ and L Abd
17. K K R. 25 Luxon ++++	0	++? empyema	Numb tingling of hands and feet, weak elevating of R arm	(III, V, X, early) 0	Bedridden, couldn't feed self, urine retention hypesthesia 3L-1S	Ptl Hands, arms, legs	Abs Bt Tr AJ L L J R, KJ =
18. J A C. 20 Germany +	0	++?	Numb feet and legs	(III, V, X, early) 0	Numb lower legs, feet and hands, can't climb stairs or descend, slapping gait	0	Abs AJ
19. M L A. 29 ? Local infect. New Guinea Lichen planus	+	0	Numb tingling of fingers	0	Numb fingers and toes, weak grip, can't climb stairs	0	Abs AJ L L J R KJ =
20. H J O. 30 Bial. =	0	--	Numbness of toes on walking	0	Tingling and numb feet and ankle, vibr sense 0 L metatarsals and toes	0	Abs AJ

NOTE.—Under Clinical-Phar. dipl. and under Cul' dipl. ++ indicates avirulent or doubtfully virulent cultures cultured. Under Cranial nerves involved, early indicates nerve involved at onset of diphtheria; it recovered to late neuritis. Under Maximum involvement—Clinical, numerals and letters indicate the cord segments involved; that is, 3C-2T signifies involvement from 3rd cervical to 2nd thoracic. Under Maximum involvement—Reflexes, degen., Ptl signifies partial. Under Maximum involvement—Reflexes Bt Tr AJ, KJ, = signifies bilateral, triceps, etc.

OF DIPHTHERITIC POLYNEURONITIS

Case No.	Residual signs and symptoms	Treatment	Dur. diph. (days)	Interval bet. diph. and neuronitis (days)	Recovery rate	Neuronitis duration (days)	Cult. diph.		Schick test	ECG changes	Spinal fluid	
							Skin.	Phar.			Max. tot. prot. (mg. %)	Coll. gold
1.	Numb fingertips, sl. weak on stairs; vibr. \pm R. and L. L ₅ -S ₁ and R. C ₇ -T ₄ ; reflex changes: AJ \pm and Bi, Tri \pm	Penc. im.	104	70	+++	150	0	-	-	+	69	+++ Paretic
2.	Numb hands and feet, slapping gait; reflex changes: Bi, Tri, KJ, AJ +; rad and ul0	Penc. im. Diph. antx.	45	30	+	85+	+	+	-	0	-	-
3.	None	Penc. local	(330 \pm) 70	75	\pm	60	Healed	-	-	0	35	0
4.	Sl. weakness R. foot, peroneals and R. hand; hypesthesia of fingers; vibr. sense \pm to 0 toes; reflex changes, none	Penc. im.	(180) 80?	30?	+++	140	Healed	-	-	+	79	+++ Paretic
5.	Sl. + fatigue of legs; reflex changes, none	Local	75	45?	+	125	Healed	-	-	0	25	+
6.	Vibr. sense \pm R. hand and foot; sl. weak R. hamstrings; reflex changes: Bi, Tri, KJ, AJ \pm	Penc. im. and local	(79) 58?	43	+++	78	0	0	-	0	80	++ Paretic
7.	Vibr. sense \pm L. foot and knee, R. lower leg; weak L. peroneals; reflex changes: AJ R. \pm ; L. 0, KJ L. \pm	Diph. antx. Penc. im.	(200) 90?	135?	+++	150	0	0	-	+	134	+
8.	Vibr. sense \pm in toes; reflex changes: AJ R. \pm	Penc. im. and local	(190) 74?	56	\pm	110	+	+	-	0	25	0
9.	R. ser. ant. and delt. paralysis and atrophy; vibr. sense \pm toes; reflex changes: L. KJ and AJ \pm	Penc. im. and local	82	77	\pm	70	Healed	0	-	0	111	0
10.	Vibr. sense + feet and R. hand, weak hamstring; reflex changes, none	Penc. im. and local diph. antx.	78	47	+	87	+	+	-	0	29	0
11.	Vibr. sense + 2nd to 5th toes; reflex changes, none	Diph. antx.	34	15	\pm	104	-	+	-	+	21	0
12.	Sl. weakness abd., arms and vibr. sense 0 to \pm lower legs and feet and 3L-4S vert.; reflex changes, none	Penc. im. and local	72	67	++	110	0	0	+	0	120	+
13.	Numb in region of ulcers on ankles; vibr. sense 0 to \pm toes; reflex changes: R. KJ and AJ sl. reduced	None	40-95	32	\pm	103	0	0	-	0	69	0
14.	Vibr. sense \pm to 0 in all toes and metatarsals; reflexes normal	Penc. local	90	60	\pm	95	0	0	+	0	42	0
15.	Sl. hyperesthesia, sl. weak R. arm; reflexes normal	Penc. im. diph. antx.	46	68	+	92	0	+	-	0	105	0
16.	Vibr. sense \pm toes, reflex changes, none	Diph. antx.	7	30	+	124	0	+	-	0	49	0
17.	Vibr. sense + toes and metatarsals; reflex changes: AJ 0	Penc. im.	170?	49	\pm early late	126	0	+	-	\pm (persists)	13	0
18.	Sl. hypesthesia to pin prick R. toes and 1st toe L. 0 to \pm ; vibr. sense R. all toes and metatarsals, reflex changes, none	Penc. im.	28?	58	+++	48	0	+	-	0	49	0
19.	Vibr. sense + to \pm all toes and fingers, reflex changes: AJ 0, KJ R. \pm and L. 0	Penc. im.	50	52	+++	68	+	0	-	0	29	0
20.	Large added walling, loss of arm movement and d. sensation R. hand; vibr. sense \pm to 0 toes and metatarsals	Diph. antx.	4	72	-	17	0	+	(-) Diph.	0	63	0

or ankle jerk, knee jerk, respectively. Under Treatment, Penc. im. or Diph. antx. indicates penicillin or diphtheria antitoxin administered by intramuscular injection. Under Dur. diph., d. ant. and Interval bet. diph. and neuronitis, sl. = less than patient's estimate; up = interval to estimate of periods; figures followed by ? indicate known periods within 1 week.

the wounds, 1 responding to diphtheria antitoxin and the other succeeding bacteriologically unproved clinical diphtheria with faucial membrane formation showing an avirulent organism on culture.

The validity of the diagnosis of diphtheria in many of these cases could be doubted except for the currently well recognized clinical picture of cutaneous diphtheria. In only 3 instances was virulent *C. diphtheriae* recovered from the skin lesions, but as has been previously stated most of these patients' lesions were clinically healed or nearly healed on arrival in the United States. In the instance of Case 17 the patient probably acquired a diphtheritic lesion of the skin of his right ear by contact in the ward of this hospital, but the diphtheroid organism obtained on culture proved avirulent. Because the lesions were healed no cultures were made on 5 patients. In all others pharyngeal and, where indicated, cutaneous or wound cultures were made. In only 1 patient (Case 10), who had pure cutaneous diphtheria, was a positive pharyngeal culture obtained, and this organism had a doubtful virulence.

Diphtheria antitoxin was found necessary in only 1 case of cutaneous diphtheria, Case 17, but it had been used overseas in 2 others, 1 of which, Case 2, was of extreme severity. It had been used in 4 of the 7 proved or suspected cases of pharyngeal diphtheria. The additional patient, mentioned previously in a footnote, developed severe late neuronitis in spite of receiving 120,000 units in treatment of a prolonged faucial diphtheria. The series is too small to comment statistically on the efficacy of diphtheria antitoxin in preventing the sequel of polynuronitis but these histories indicate that such treatment will not uniformly prevent this sequel.

The clinical course of 20 cases of polynuronitis in this series follows the pattern of the Guillain-Barré syndrome with certain exceptions. There was an interval of 30 to 75 days, excluding Case 7, between the onset of the skin ulcers and the neuronitis. In many instances the ulcers

were clinically cured, although in only 2 instances were they completely healed at the onset of neurologic signs or symptoms. The onset of the polynuronitis in 17 cases was evidenced by paresthesias and numbness of the hands and (or) feet followed, in a few days to 2 weeks, by motor signs consisting chiefly of foot drop and (or) weakness in lifting the arms. In 3 cases a stumbling gait or weakness in climbing stairs were the presenting symptoms. In contrast to the Guillain-Barré cases no patient complained of any significant pain. The advance of the process was generally slow for several weeks and then the motor symptoms abruptly increased.

Contrary to the usual involvement of cranial nerves, especially the seventh nerve, in the Guillain-Barré syndrome, only 5 of these cases showed transient cranial nerve involvement and only 3 had mild facial nerve involvement. The first and second sensory divisions of the fifth cranial nerve were involved in all 5 cases. In 2 of the 7 cases with faucial diphtheria, transient cranial nerve signs had been present during the acute phase. Four additional cases of early cranial nerve involvement failed to develop any signs of cranial nerve disease during the late form of neuronitis.

An unusually mild and prolonged course of the proved or suspected diphtheritic infection was characteristic for most of these patients who later developed polynuronitis. The duration of the infection was estimated from 45 to 104 days with the majority persisting about 75 days. Of the cases of pure pharyngeal diphtheria, 3 showed prolonged courses of active infection, namely 32, 46 and 28 days, repeated administration of antitoxin being necessary in Cases 11 and 15. Cases 16 and 20 had normally short courses, with prompt response to antitoxin. Liebow¹² and Dudley⁵ suggest that such mild diphtheritic infections fail to raise the concentration of antitoxin in the blood to a level which would prevent prolonged absorption of toxin or reverse the Schick

reaction in some cases. Only Cases 12, 14 and 18 of our series demonstrated a positive Schick reaction in the late stages of the disease. Case 18 was a patient with clinically characteristic pharyngeal diphtheria from whom it was impossible to culture virulent diphtheria bacilli. He received penicillin but no antitoxin. Cases 12 and 14 were cutaneous diphtheria. It has also been suggested that certain of these strains of diphtheria may differ from others more commonly encountered in the United States, in their tendency to produce mild prolonged infections.

The severity of the cases is indicated by symbols of \pm , $+$, $++$, $+++$, $++++$, indicating mild, moderate, severe and very severe attacks. Cases 14 and 20, classified as \pm , had definite but minimal symptoms of paresthesia and weakness (of legs only in Case 20), had no loss of reflexes but loss of vibratory sense in the toes. Such cases might be readily missed unless the observer was alert to the characteristic signs and symptoms of the disease. Cases 1, 2, 4, 6, 7 and 17, classified as $++++$, were all bedridden and required assistance in feeding and turning their bodies. No patient had interference with respiration, nor were cerebellar signs or mental abnormalities observed. Almost all cases demonstrated widespread loss of the deep tendon reflexes of the arms and legs and frequently of the abdominal and cremasteric reflexes. Cases 14 and 20, although otherwise characteristic, at no time demonstrated any loss of reflexes. A partial electrical reaction of degeneration of certain muscles was temporarily demonstrated in all grade $++$ to $++++$ cases and persisted in the right deltoid and serratus anterior in Case 9.

The recovery was often abrupt, which is less frequently observed in reports of the Guillain-Barré syndrome in which occasional cases are reported as persisting for 3 years. Recovery is generally complete with rare residuals of weakness, atrophy or sensory damage. The rate of recovery is indicated in Table 1 by 4 symbols from \pm , a slow recovery rate, to

$+++$, indicating return of function from a completely bedridden helpless patient to an active ambulatory state within 3 weeks.

The criteria for complete recovery are difficult to define. When function and sensation returned to a constant state for 1 to 2 months the patients were adjudged as recovered. The duration of the disease varied from approximately 50 to 155 days with the exception of Case 20, the mildest of the series, which lasted 17 days. There were only 5 patients who had exhibited a complete areflexia and then recovered normal reflexes after 3 to 7 months observation, namely Cases 3, 5, 11 and 15 within 4 months and Case 1 only after 7 months. Cases 14 and 20 at no time showed loss of reflexes. Case 2 was not followed after the 3rd month being transferred to another hospital while still exhibiting considerable involvement but in a recovery phase. Nine patients demonstrated slight muscular weakness chiefly in the peroneal, hamstring and hand muscles. Only in Case 9 was persistent weakness and atrophy of marked degree noted after apparent recovery, and this was in the right serratus anterior and deltoid muscles (Figs. 1 and 2). Subjective paresthesia and numbness were persistent in only 2 cases, excluding Case 2, but slight hypesthesia to pain and touch in fingers and toes was present in 4 cases. The most constant residual finding, exclusive of hypesthesia in or adjacent to the diphtheritic skin ulcer scars, was a reduced perception of tuning fork vibration in the fingers and toes. In contrast to the early stages, in the late recovery phase the pattern of sensory diminution did not follow segmental or characteristic major peripheral nerve distribution. "Glove" or "stocking" type anesthesia was common and suggested distal involvement of peripheral nerves. The irregular involvement of different phalanges excluded hysterical origin. Occasionally there was vibratory hypesthesia of the spinous processes of the lumbar and sacral vertebrae, in the long bones of the extremities and the ilia.

Frequently hypesthesia of the soles and

palms accompanied the recovery and persisted. This may have been partially due to the hyperhidrosis commonly observed in these patients.

fatality from respiratory paralysis or a complicating pneumonia could occur in this type of case.

The spinal fluid protein in Guillain-



FIG. 1.—Case 9. Anesthetic, pigmented scars of healed cutaneous diphtheria.



FIG. 2.—Case 9. Residual serratus anterior and deltoid muscle paralysis resulting in winging of right scapula.

In contrast to a mortality of 14 to 42% in certain reports of the Guillain-Barré syndrome, no fatalities have been reported from these postdiphtheritic cases, although the extreme paresis suggests that

Barré syndrome is characteristically elevated from 70 to 400 mg. per 100 ml., generally without an increase of cellular elements. In Liebow's¹² series the spinal fluid protein varied from normal to

125 mg. per 100 ml., with the majority varying from 60 to 80 mg. per 100 ml. Only 9 of our cases demonstrated spinal fluid total protein content of 60 mg. per 100 ml. or more, 2 of which were 120 and 134 mg. per 100 ml. Five of our series demonstrated a paretic (first zone) type of colloidal gold curve and 1 case a mid-

myocarditis found in these patients was not a result of diphtheria but a complication of the neuronitis syndrome. Myocarditis with electrocardiographic abnormalities has been reported in Guillain-Barré syndrome and late myocarditis was noted in 1 of Liebow's¹² cases and has been mentioned in other personal communica-

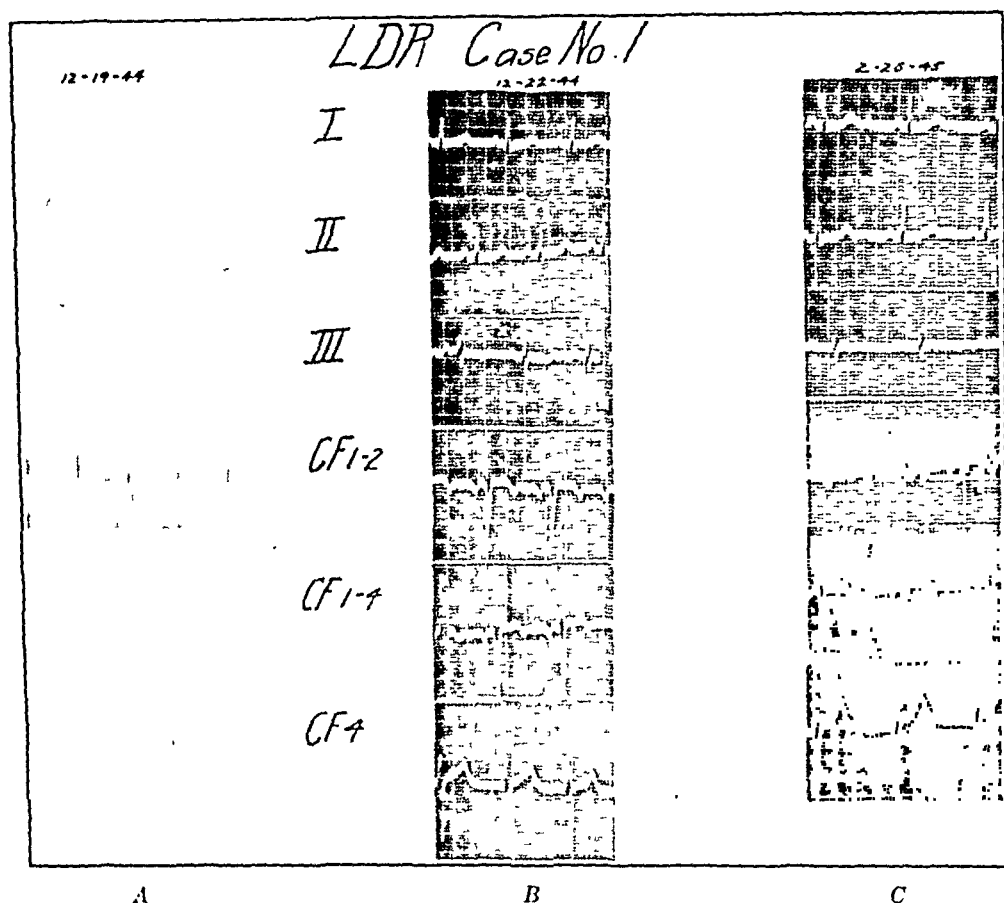


FIG. 3.—Electrocardiograms of Case 1. A, Records of Dec. 19, 1944, taken during advance of polynuronitis. Depression of S-T segment in Lead I and Lead II with elevation in Lead CF₁₋₂ (chest lead in 2nd right intercostal space). T wave depressed in Leads I and II and inverted in Lead III and upright in Lead CF₁₋₄ (chest lead in 4th right intercostal space). B, Records of Dec. 22, 1944, show marked improvement with no significant change in the clinical state. C, Records of Feb. 26, 1945, show further improvement in S-T segment and T wave changes in Leads I, II, III and CF₁₋₂ and CF₁₋₄. Clinical recovery was well advanced at this time.

zone type. The total protein content, when elevated, increased in proportion to the severity of the symptoms. A convalescent elevation is not uncommonly observed in Guillain-Barré syndrome and was present in Case 20.

Although we have no early electrocardiograms except in Case 17, it is our belief that with the exception of this case the

tions. Tachycardia was present in 7 of our cases and also definite but transient electrocardiographic changes in 5 of this series (Figs. 3 and 4). In Case 17 electrocardiographic abnormalities persisted with little change from the onset of the acute diphtheria through complete recovery of the neuronitis.

No evidence of renal damage was noted.

Two instances of acute hepatitis (Cases 7 and 20) occurred during the height of the illness. These may have been coincidental attacks insofar as acute hepatitis was prevalent at the time in the area from which these patients were evacuated.

Drug therapy, including thiamine chloride, is reported as worthless and no change in the clinical course was made by daily

motor function in these patients. Case 6, who was completely bedridden and unable to lift his head or extremities for nearly 3 weeks, demonstrated no muscle atrophy and approximately normal use of his arms, legs and trunk within 10 days of the first time he was able to stand upright. This would be a remarkable recovery for any bedridden patient, but for such a

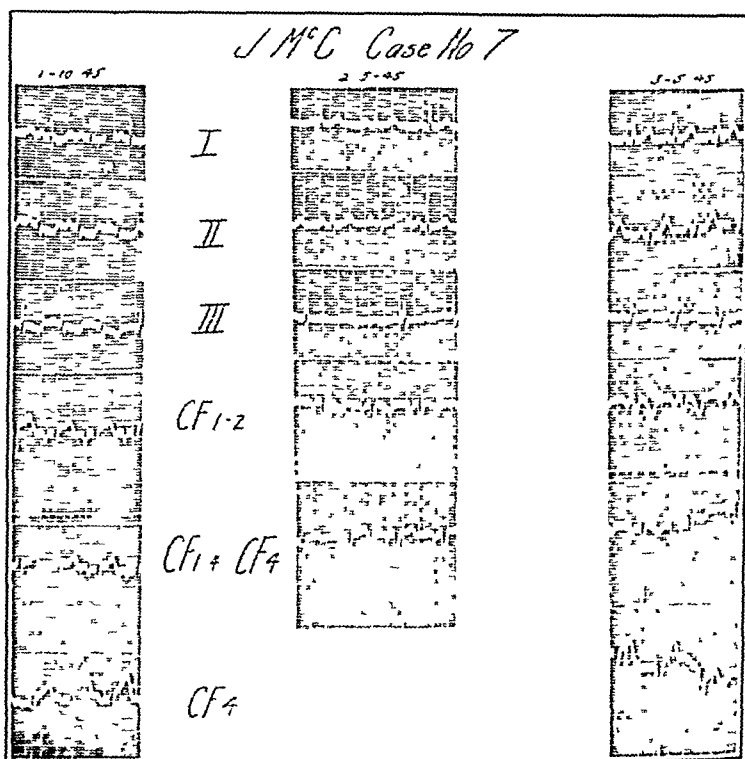


FIG. 4.—Electrocardiograms of Case 7. Records of Jan. 10, 1945, taken during advancing phase of polyneuritis. Depression of S-T segment in Leads I, II and III with elevation in Leads CF_{1-2} , CF_{1+4} and CF_4 . T wave depression in Leads I and II, inversion in Lead III and upright in CF_{1-2} and CF_{1+4} . Records of Feb. 5, 1945, taken during severe phase of the disease. S-T segments are isoelectric, T_1 and T_2 are upright, T_1 isoelectric and T_2 inverted. Records of Mar. 5, 1945. Taken in early recovery phase, shows return to normal pattern with T_1 now upright.

doses for 2 to 4 weeks of 100 mg. thiamine chloride intramuscularly in 4 cases during the advancing phase of the process. It has been suggested that prostigmine may be valuable in the paralytic phase of this disease, but it was not used in this series of patients.

Physiotherapy in the form of daily massage and graded exercises results in prevention of atrophy and the loss of

patient with generalized paralysis it was a strong indorsement of this form of treatment.

The following is a more detailed review of 3 cases in the series, the first 2 being a mild and a severe attack following cutaneous diphtheria, the third being a severe one in which virulent diphtheria bacilli were suspected of being present in an empyema wound, but never demonstrated

Case Histories. CASE 6. H. A. O. (Fig. 5.) This 24 year old enlisted man developed a vesicular dermatitis, generalized, while in combat and living on C & K rations, in the Southwest Pacific theater of military operations in mid-November 1944. The dermatitis cleared with local treatment and he returned to duty in 2 weeks, but about Dec. 6 1944, about 15 ulcerated lesions appeared on both lower legs, ankles and feet. He was hospitalized with a mild fever (maximum of 100.4° F.) and was

ulcers had started to heal. About Jan. 18, 1945, he noted numbness and tingling of all his finger tips and within 24 hours similar sensations in the toes and "balls of the feet." He was admitted to Baxter General Hospital on Jan. 23, 1945, with about 10 partially healed ulcers varying from 5 mm. to 4 cm. in diameter and with the above-mentioned symptoms of numbness and tingling in his hands and feet and a tendency of his legs to buckle under him when he walked. Gentian violet solution was applied to the ulcers

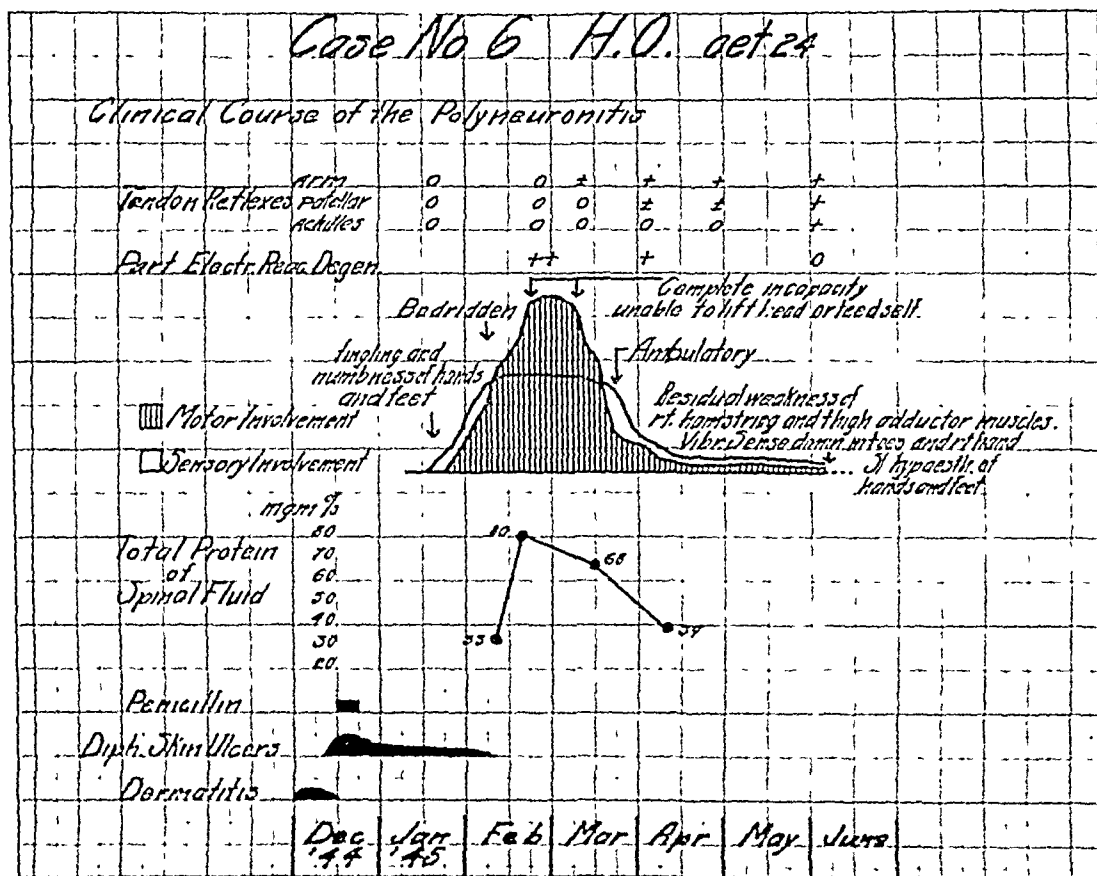


FIG. 5.—Case 6. Chronologic diagram of clinical course and spinal fluid protein changes.

treated with sulfadiazine orally, penicillin intravenously and local saline and penicillin moist dressings on the lesions. Although the skin lesions were characteristic diphtheritic ulcers, round or oval with sharply demarcated borders and grayish brown membranes which later crusted, *C. diphtheria* was not isolated by culture. No diphtheria antitoxin was administered.

He was evacuated to the continental United States in early January 1945 as a litter patient by which time many of the

and dry dressings were used and the ulcers healed completely within the next 4 weeks, with smooth anesthetic surfaces and heavily pigmented areolae.

A neurologic examination on Jan. 25, 1945, revealed hypesthesia, hypalgesia and diminished temperature sensation in both hands and lower legs, and weakness of the muscles of the shoulders, arms and legs, and especially the posterior deltoids, muscles of the hands and the hamstring and peroneal groups. There was reduced vibratory sense

of the bones of the hands, forearms, feet and lower legs. Biceps, triceps, patellar and Achilles tendon reflexes and the abdominal and cremasteric reflexes were absent bilaterally. There was no cranial nerve involvement. The leukocyte blood count was 11,600 per c.mm. (9% eosinophils). The spinal fluid on January 27 revealed 1 cell per c.mm., 33 mg. per 100 ml. total protein, a heavy trace of globulin and a colloidal gold reading of 2111110000. The Schick test was negative.

His condition remained stationary with the exception of a diminution of the numbness and tingling of the feet and hands between February 24 and March 14. Studies of sensory changes in this period illustrate a segmental distribution of hypesthesia, hypalgesia, diminished temperature sensation, vibratory sense and proprioception as in Figure 5, namely C_4 to T_4 and L_4 to S_1 on the right and C_4 to T_3 and L_4 to S_1 on the left. Asterognosis and atopognosis existed in the hands and feet. Muscle studies revealed

SENSORY EXAMINATION (Chart on anatomical figures): *Rt Foot, Rt L Hands*
 Light touch \pm Pain \pm Temperature \pm Vibratory \pm Position \pm Stereognosis \pm Topognosis \pm

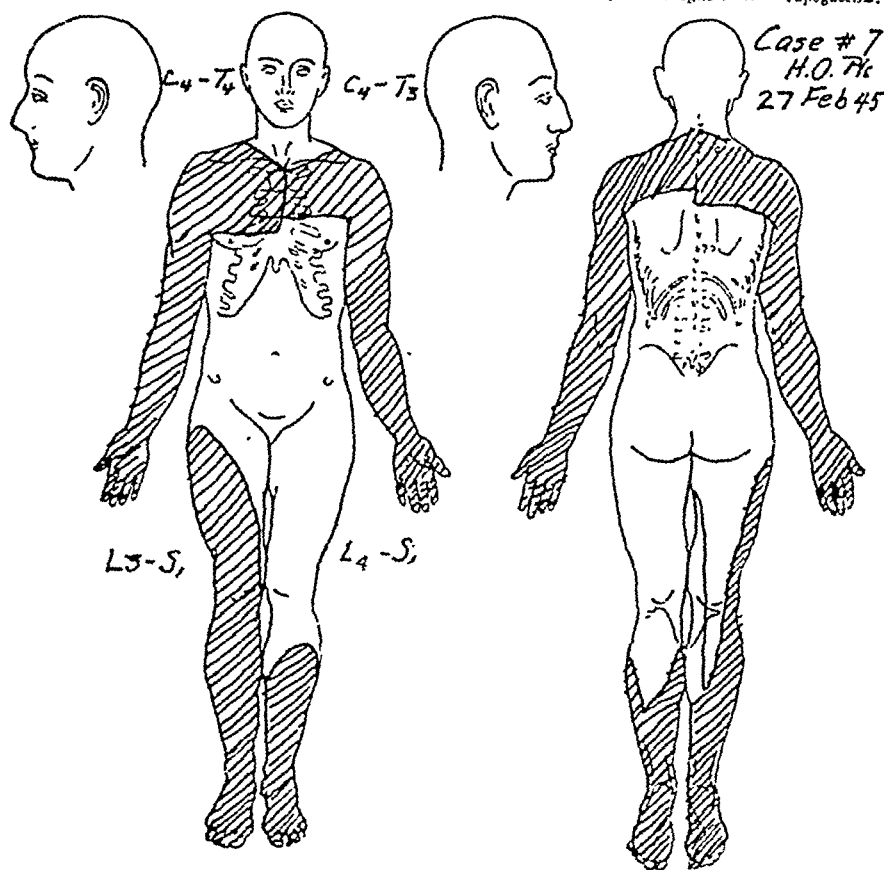
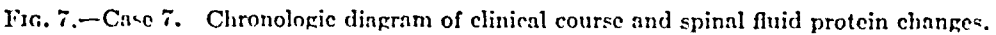


FIG. 6.—Case 7. Pattern of hypesthesia and hypalgesia

His sensory symptoms remained unchanged but the weakness slowly increased and by February 12 he was unable to stand unassisted or to lift his arms to comb his hair. By February 24 he was unable to elevate his trunk, head or legs, to turn himself in bed or to lift his forearms to feed himself.

reduced power of all muscle groups of the trunk and extremities and a "poor" rating (inability to move against gravity) of the posterior deltoids, all hand muscles, hip rotators and gluteals, bilaterally, and of the left hamstring, peroneal groups and gastrocnemius and of the right middle and lower

On April 7 the tendon reflexes of the upper and lower extremities with the exception of the right Achilles tendon reflex which was absent, had returned although they were faint. There was demonstrable weakness of the right thigh adductors and hamstring groups and hypesthesia of the entire right foot and left second to fifth toes as well



Improvement continued slowly with toe drop and slapping gait and slight general leg weakness until about May 1, but paresthesias had completely disappeared by April 22. On return from furlough on June 4 his spinal fluid showed 2 cells, 39 mg. per 100 ml. total protein, a negative colloidal gold test, and a trace of globulin. All tendon reflexes and superficial reflexes were present but the Achilles reflexes showed diminished force. There was slight hypesthesia of the toes of the right foot and in and adjacent to the scars of the ulcerative lesions, the dorsum of the left foot and toes

On March 15 the spinal fluid showed 3 cells, 68 mg. per 100 ml. of total protein.

and anterior lower two-thirds of the left lower leg. Vibratory sense was diminished in all toes and in the right and left fourth and fifth metatarsal bones. There was no demonstrable muscle weakness and electrical reactions were uniformly normal.

Thiamin chloride in doses of 200 mg. per day was given intramuscularly without apparent effect in the advancing stages of the disease. Massage and passive and active

abruptly developed shortness of breath. This became increasingly severe in the next 24 hours until he was hospitalized at which time the presence of a massive spontaneous hemothorax on the left was determined by thoracentesis. No significant trauma preceded the hemothorax and no cause was found for it at later investigations. After a second tapping he developed fever and empyema was diagnosed. On April 19 he

SENSORY EXAMINATION (Chart on anatomical figures): *Hyparsthesia*

Light touch. \pm Pain. \pm Temperature. \pm Vibratory. *N*-Position. *N*-Stereognosis. *N*-Topognosis. *N*-

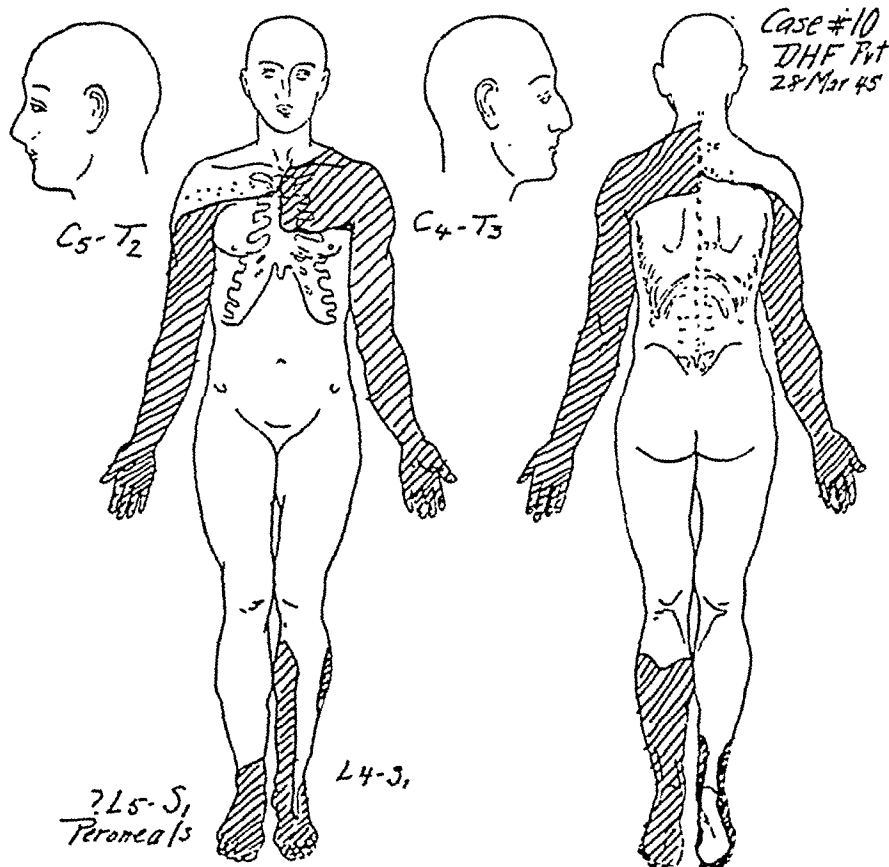


FIG. 8.—Case 10. Pattern of hypesthesia and hypalgesia.

exercise were used through the entire course in progressive degrees. The constant attention of the physiotherapist was probably responsible for the rapid return of strength and for complete absence of muscle atrophy after the process had started to recede.

CASE 7. J. McC. (Figs. 6 and 7.) This patient, age 26, gave a history of onset of his present illness in January 1944 while stationed in Alaska, at which time he

was transferred to Hammond General Hospital, California, and on May 21 a thoracotomy was done with resection of the eighth and ninth ribs on the left. Following this his wound was irrigated daily with Dakin's solution, and upon admission to this hospital the empyema cavity contained approximately 180 cc. of fluid. For approximately 1½ months before admission the size of the space had not altered appreciably.

His general condition upon admission to this hospital was good and he was ambulatory. Because the space was not obliterated by October 23, a Schedé thoracoplasty, with resection of the fourth to ninth ribs with complete unroofing of the space was carried out. The patient received penicillin postoperatively until November 12 at which time the space measured approximately 50 ml. The space continued to reduce in size for the succeeding 4 weeks. His general condition was satisfactory, but about No-

dency toward ulceration of the granulation tissue and skin about the opening of the wound. The patient continued to run a septic type of fever and have pain in his chest. On December 13, when increased drainage of the empyema was noted and the space reopened, it was found to hold approximately 50 ml. of fluid. It was now noted that a very definite cellulitis of the wound had developed and the infection was indolent, characterized by the production of considerable purulent material

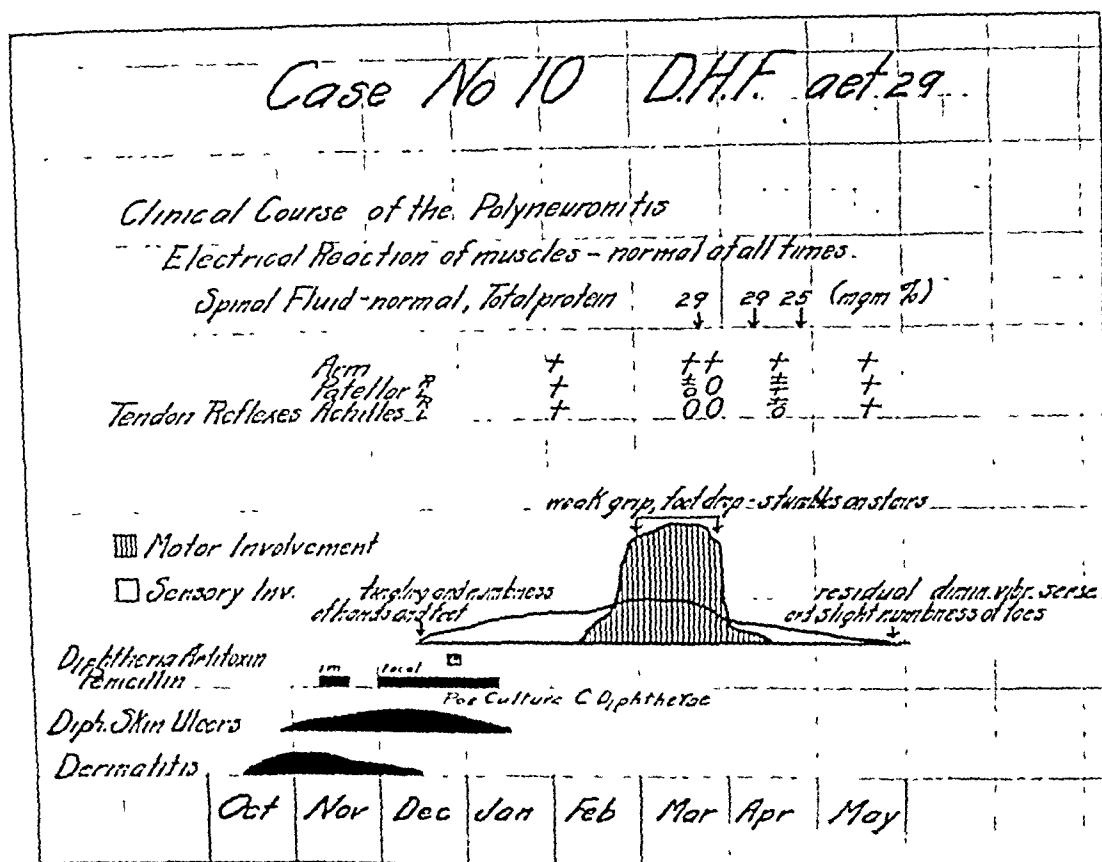


FIG. 9

vember 24, the patient began to complain of pain in the left chest and a friction rub was noted. Examination of the wound was negative and there was no evidence of a bronchopleural fistula. In December the space contained about 10 ml. of fluid and there was considerable amount of granulation tissue about its opening. About this time the patient began to run a fever of 100° to 101° F. He complained of pain around the opening of the wound and the wound itself became inflamed with a shaggy dirty coating of fibrin upon it, and a ten-

shaggy fibrin coating on the pleural surfaces, and a tendency toward ulceration of the skin borders around the wound. Because of the chronicity of the infection, material from the wound was stained, cultured and injected into a guinea pig in search of tubercle bacilli but none were found. Because of the cellulitis of the wound Roentgen ray radiation was given and there was some moderate improvement of the external appearance of the wound. Penicillin was given both systemically and locally

without much alteration of the course of the disease.

About the end of December 1944, he rather abruptly developed numbness and paresthesias, and hypesthesia was demonstrated in the distribution of the fifth cranial nerve, and the ninth to eleventh thoracic nerve segments, especially on the right, the fifth to eighth cervical segments and the first thoracic segments on the right and left. These changes involved the lower neck and portions of the arm, the upper chest and a band around the mid-chest and upper abdomen. This area showed hypesthesia, hypalgesia and diminished temperature sensation. The symptoms and signs increased in severity and about Jan. 10, 1945, he developed diplopia on looking towards either side, due apparently to an involvement of the right and left sixth cranial nerves. This cleared in a period of about 1 week. On January 18 there was slight paralysis of the left twelfth cranial nerve noted by the tongue protruding to the left, and there was a slight persistent weakness of the right external oblique ocular muscle.

From January 1 to 20 he complained of tinnitus in his right ear with slight impairment of hearing on that side indicating involvement of the right eighth nerve. He had a moderate headache during January. The process advanced through the middle and end of January and by February 1 there was difficulty in swallowing indicating involvement of the motor roots of the fifth and possibly tenth cranial nerves. There was difficulty with phonation and bilateral hypesthesias of the face. Whereas the deep muscles and cutaneous reflexes had been normal on January 18, all tendon reflexes in the arms and legs including knee jerks and ankle jerks and all abdominal, cremasteric and corneal reflexes were found to be absent. He had an atypical Babinski reflex on the right. Weakness of the legs had developed most notably in the quadriceps, peroneals and the hamstring muscles, and there was hypesthesia and paresthesias from D-10 to S-2.

By February 21 the weakness had increased especially in the shoulders and arms, and there was some atrophy of the interossei muscles. His spinal fluid, which earlier was not abnormal, at this time showed a total protein of 53 mg. per 100 ml. About February 20 he developed an acute hepa-

titis as evidenced by jaundice, light stools, malaise and nausea with slight rise of temperature over his previous level.

The jaundice and laboratory evidences of hepatitis disappeared within 2 weeks but throughout the last week of February and all of March he was completely bedridden being unable to feed himself, lift his head or turn his body. Throughout the entire period he had demonstrated a moderate fever. His Schick test was negative and cultures deep from the chest wound showed avirulent diphtheroid organisms. In spite of this fact the use of diphtheria antitoxin was indicated in view of the nature of the wound and the neuronitis which had developed. On March 24, 20,000 units were administered and as a result of this his temperature returned to normal in 3 days and remained unelevated. There was a marked improvement shortly demonstrated in the character of the wound and rapidly diminishing discharge from the empyema cavity which spontaneously healed.

About April 1 he abruptly showed improvement in the neuronitis, although as early as the middle of March there was a return of sluggish knee jerks. The total protein of the spinal fluid was 117 mg. per ml. on April 5. By April 14 he showed general improvement in strength, and was able to stand and walk with only moderate assistance. Improvement continued rapidly and about May 20 he was aware of no significant weakness. However, the knee jerks were diminished as was the right ankle jerk and the left ankle jerk was absent. There was definite diminished vibratory sense in both feet and the right patella. His spinal fluid protein on June 8 still showed 60 mg. per 100 ml.

The electrocardiogram on January 10 showed depression of S-T intervals and flattening of T₁ with inversion of T₂ and T₃. The electrocardiograms of early February, March and April had returned to a normal pattern.

At the time of his discharge, July 4, 1945, he presented only slight weakness of the dorsiflexors of both feet and a slight consciousness of a slapping gait on the left due to foot drop when walking rapidly. His ankle jerks were very sluggish even with reinforcement but the knee jerks were present although the left was faint. He had a clean-cut Babinski reflex, although the right

plantar reflex occasionally showed an atypical dorsiflexion of the great toe. There was a loss quantitatively of vibratory sense of all the toes and in the metatarsal bones and the internal malleolus on the left. He had gained 35 pounds from his lowest weight, as of April 1, and his general condition was excellent. There was no evident muscle atrophy. His general muscular strength was excellent with the exceptions noted above.

CASE 10. D. H. F. (Figs. 8 and 9.) This 29 year old infantryman developed a mild dermatitis of his lower legs in October 1944 when in the Southwest Pacific theater of operations. Ulcerative lesions appeared on both lower legs, ankles and feet and 1 on each wrist by November 1. They increased in number and size requiring hospitalization on December 1. Virulent C diphtheriae was cultured from an ulcer and a diphtheroid organism of doubtful virulence from the throat on December 9. Penicillin was administered intramuscularly for 6 weeks beginning December 10 in amount of 120,000 units per day, and as an ointment on the skin lesions. On December 27, 40,000 units of diphtheria antitoxin were given intramuscularly. The skin lesions slowly healed and were smoothly epithelialized by Feb. 15, 1945.

About Dec. 15, 1945, slight numbness and tingling of the toes and numbness of the feet and ankles was first noted and about January 12 the numbness had extended up the left lower leg and there were paresthesias and numbness of both hands and wrists. This was not troublesome and the history was elicited only on specific questioning. He had moderate difficulty in focusing his eyes on reading from Dec. 15, 1944, to Feb. 1, 1945.

Physical examination on February 10 revealed slight hypesthesia to pain, touch and temperature from the fourth lumbar to the first sacral dermatomes and slight reduction of vibratory sense in the toes. There was slight weakness of both deltoid muscles. All superficial skin and deep tendon reflexes were normal and the spinal fluid was normal, namely 29 mg. per 100 ml. of total protein, 1 cell per c.mm. and a normal colloidal gold determination.

About February 15 he first noted weakness of the grip of his hands, a slapping gait with foot drop and difficulty in ascend-

ing or descending stairs. This weakness was observed while the patient was home on furlough and on his return to the hospital on March 25 improvement was evident. Examination on March 28 revealed an absent right patellar reflex and absent Achilles reflexes bilaterally with weakness of both quadriceps femoris, hamstring and peroneal groups and the extensor muscles and intrinsic muscles of the hands. There was hypesthesia of both hands and wrists and of the third lumbar to the first sacral segments bilaterally except for hyperesthesia of the right sole.

The patient's condition improved slowly. Repeated spinal fluid examinations were normal. By April 20, 1945, he complained only of slight numbness of the feet which were hypesthetic and slight weakness of the left leg. The patellar and left Achilles reflexes were present but faint and the right Achilles reflex was absent. At the time of his discharge on May 28, 1945, he had no complaints but there was slight weakness of the left hamstring muscles, vibratory sense lost in the right great toe and diminished vibratory sensation in the phalanges of the other toes and the right fingers and wrist bones. All tendon reflexes were normal at this time.

Summary and Conclusions. Twenty cases of late polyneuritis are presented following proven or suspected cutaneous and faucial diphtheria. This syndrome differs from the early diphtheritic neuritis commonly observed. Approximately 20% of all cases of cutaneous diphtheria acquired in the South Pacific theater of operations, observed at the hospital in which these studies were made, developed polyneuritis.

The clinical picture and spinal fluid findings closely resemble the Guillain-Barré syndrome with the exception that pain was not a symptom in any case.

The initial symptoms occurred generally from 1 to 3 months after the onset of the diphtheria. These symptoms were commonly numbness and paresthesias of the hands and feet. At the height of the illness, 5 of the patients were completely incapacitated, but several presented only minor sensory and motor disturbances

and the neurologic changes would have been overlooked without careful interrogation and physical examination.

Partial electrical reactions of degeneration of the involved muscles were obtained in all the severe cases.

The segmental character of motor and sensory involvement, generally C₁ to T₂ and L₁ to S₁ during the early and most severe stages of the disease, receded to signs of irregular distal peripheral neuritis. Recovery was generally abrupt and the common residual signs after 4 to 7 months of observation were absent Achille and patellar reflexes and diminished vibratory sense in the toes. Only 1 patient demonstrated marked residual loss of motor power, localized in the right serratus anterior and deltoid muscles.

Muscle are and passive and active exercises throughout the course of the disease apparently hastened the return to normal motor function and prevented muscle atrophy.

It is believed that the frequency of this syndrome following current diphtheria epidemics, both overseas and in the United States, as contrasted to its rarity in this country in recent years, may be attributed to prolonged courses of the diphtheria infection. It has been suggested that the infecting bacteria in this type of case have poor antigenic properties, and this concept is supported by the persistence of a positive Schick test in 2 of the patients.

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NEEDLE BIOPSY OF THE LIVER

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NEEDLE biopsy of the liver has gained popularity slowly. The first recorded puncture was performed in 1833 by Stanley² to drain an abscess. Probably the first puncture for the purpose of biopsy was done by Lucatello¹⁰ in 1895. Although used not infrequently in Europe, the procedure was for the most part neglected by American workers until the advent of widespread epidemic hepatitis in World War II.

From our experience with about 100 biopsies we believe that frequent use of this procedure is justified. The first 79 of these biopsies have been analyzed to estimate the chances of obtaining satisfactory material for microscopic examination as well as to determine the diagnostic value of such a procedure.

There are 2 general methods of doing needle biopsy of the liver. In 1 a very small bore needle is introduced into the liver with or without the administration of an anesthetic and a few cells are aspirated with a small syringe. These are expressed onto a slide, smeared and examined directly and again after staining, usually with a Romanovsky dye. Although this method is safe, its proponents^{4,5,7,16,18} freely admit that the study of stroma and architecture is impossible with the specimen obtained. They do state, however, that frequent diagnoses are possible.

The other method employs a larger needle which yields specimens of tissue large enough for the preparation of paraffin section. This was originated in 1923 by Bingel and Olivet, quoted by Olivet,¹¹ and

extended by Olivet¹¹ in 1926. This method has been attended by a certain degree of risk. Olivet¹¹ reported 2 deaths from hemorrhage in 140 biopsies. In 1939 Inversen and Roholm^{8,13} did 160 biopsies on 114 patients using the large needle technique through an intercostal approach in the posterior axillary line. Satisfactory specimens were obtained in 77.5% of the cases and 2 patients showed clinical signs of moderate to marked intraperitoneal hemorrhage. However, the patients died later and at autopsy it was thought that the deaths were not due to hemorrhage.

Baron,¹ who reported the first liver biopsies in the United States, used a technique similar to that of Iversen and Roholm except that the liver was approached from below the right costal margin anteriorly. In 49 biopsies on 36 patients he had 1 death from hemorrhage; in 1 of the patients he obtained a small piece of colonic mucosa without apparent ill-effect to the patient.

More recently, Tripoli and Fader¹⁷ reported 14 cases done with the Silvermann¹⁵ needle from the anterior subcostal approach without complication. Dible, McMichael and Sherlock³ performed biopsy on 126 cases of acute hepatitis using the technique of Iversen and Roholm with 1 death due to intraperitoneal hemorrhage. Two other patients showed signs of intraperitoneal hemorrhage within 24 hours of the procedure but did not die. Subsequently, 2 other patients died, 1 having acute hepatic necrosis as shown at autopsy and the other having a large amount of blood-stained ascitic fluid.

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They concluded that "the technique cannot be recommended as a routine procedure." Kalk and his collaborators² reported biopsies obtained by combined laparotomy and liver puncture with a 2 mm. cannula having a saw-toothed edge. There were no complications in 123 patients studied over a 2 year period.

Hoffbauer,⁴ on a basis of experience gained in the performance of 65 biopsies, recommended the use of a modified Silvermann needle and found the procedure safe in the presence of a large and easily palpable liver but did not advocate it for the normal sized liver. More recently, however, 1 severe hemorrhage has been reported from his Clinic.⁴ Raby¹² added a case of fatal hemorrhage in his series to bring his collected total to 7.

and more recently the Roth-Turkel,¹⁴ consistently good results have been obtained. The Roth-Turkel needle (Fig. 1) consists of an outer trocar with stylet and an inner hollow needle with small saw-teeth at the end. The trocar is advanced to the surface to be examined and the stylet removed. The inner needle is introduced and slowly advanced and rotated to cut out a core of tissue about 2 mm. in diameter. The end is moved slightly to free the core and the needle withdrawn as negative pressure is maintained with an attached 20 cc. syringe. A cylinder of tissue 0.2 cm. by 2 or 3 cm. is usually obtained.

The procedure is done at the bedside without preparation except for mild sedation for apprehensive patients. The liver

TABLE 1. TOTAL NUMBER OF BIOPSIES DONE EACH YEAR WITH THE PERCENTAGE OF SUCCESSFUL ONES (1 of the 5 in 1943 After July 1)

Years	1939-40	1941	1942	1943	1944	1945	Total
Biopsies	11	6	8	5	11	38	79
Successful	6	3	6	5	10	31	68
% successful	54.5	50.0	75.0	100	90.8	89.5	86.1
Autopsied	3	2	3	0	3	7	18

In the Cleveland City Hospital from the latter part of 1939 to July 15, 1945, 88 needle biopsies of the liver were done on 79 patients. Nine of these were done postmortem, 4 antemortem with peritoneoscopy control and 67 antemortem without peritoneoscopy. The procedure was done by several different physicians and the pathologic material was studied by different pathologists. For more accurate evaluation the study has been divided into 2 periods. The first part, extending from 1939 to July 1, 1943, includes 26 biopsies which were done without special instruments and with only a moderate degree of success—61.5% (Table 1). The technique during this time consisted of introduction of a large bore needle into the liver and aspiration with a syringe. Specimens consisted of powdery flecks of tissue which were fixed in Schaudinn's solution centrifuged, and sectioned. During the second period from July 1, 1943, to July 15, 1945, by use of the Silvermann¹⁵ needle

is palpated and unless there is a definite nodule to be entered, a spot a few centimeters to the right of the xiphoid process and below the costal margin is selected. The skin is cleansed and prepared with alcohol and iodine. A sterile drape and gloves are used. The skin is infiltrated with 1 or 2% procaine and the infiltration is carried down to and including Glisson's capsule if possible. The skin is incised with a bistoury and the biopsy accomplished, the needle usually being directed upward and to the right toward the center of the liver. The activities of the patient are not limited as a result of the procedure.

There are certain risks which the procedure entails. Possible hazards include excessive bleeding into the peritoneal cavity or abdominal wall, perforation of the large or small intestine or gall bladder, introduction of infection or spread of localized infection into the general peritoneal cavity, and injury to nearby viscera such as the kidney, adrenal gland, or pancreas.

In our series there were no serious consequences. Nine patients complained of abdominal pain for varying periods after the procedure, the usual period being for 1 or 2 days, but 2 patients continued to complain for 5 days. One patient had moderate epistaxis following the procedure. In 1 case peritoneoscopy was done the day following the biopsy, and except for a very few small particles of old blood in the peritoneal cavity no evidence of damage was seen. In 18 autopsies no evidence of serious damage was found.*

four patients are known to have died, in 18 of which autopsy was performed.

The relative frequency of each biopsy diagnosis is shown in Table 2. These diagnoses were reported by 3 different pathologists and the diagnoses are unaltered, although cases were reviewed. There is a striking difference in both the diagnostic and unsuccessful biopsies before and after introduction of the special needles. The diagnosis of carcinoma is by far the most common and includes all slides in which the diagnosis of malignant

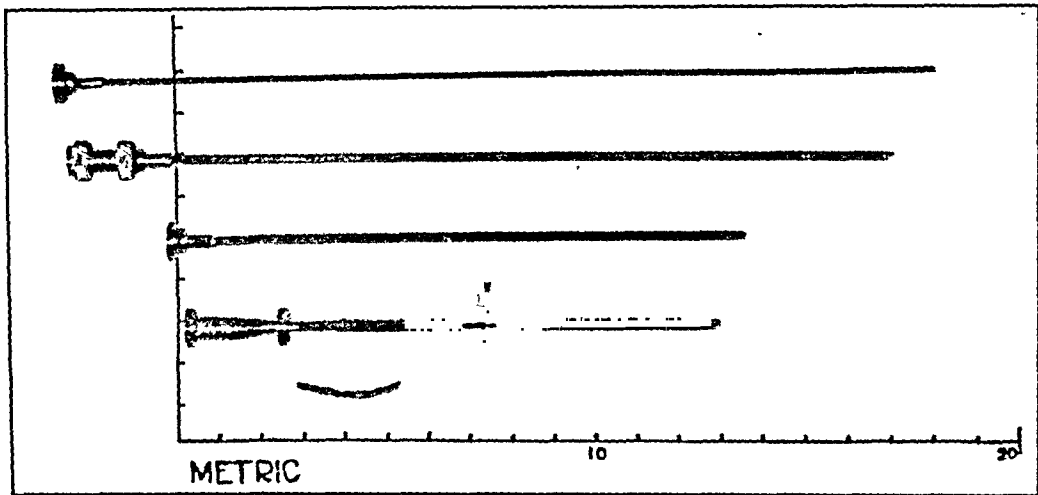


FIG. 1.—Roth-Turkel needle with gross specimen obtained. Scale is in centimeters.

Table 1 shows the number of biopsies done each year with the degree of success. Four of the liver biopsies done in 1943 were after the introduction of the special needles. The concomitant increase in utilization and percentage of success is easily seen. Biopsies were considered successful when an amount of liver tissue was obtained which was large enough for pathologic study. The period in 1945 includes only those up to July 15, 1945. The group included 52 men of whom 46 were white and 6 Negroes, and 21 women of whom 14 were white and 7 Negroes. Ages ranged from 21 to 79 years. Forty-

neoplasm was made. The most frequent of these was "partially differentiated adenocarcinoma metastatic to liver," but some were less specific—"undifferentiated carcinoma" or "malignant tumor" of the liver. The diagnosis of fatty metamorphosis was usually a secondary one in a case of tumor or cirrhosis but in a few instances it was the only diagnosis. A report of "consistent with Laennec's cirrhosis" usually stated periportal or focal fibrosis and chronic periportal hepatitis consistent with Laennec's cirrhosis if representative of the entire liver. An unequivocal diagnosis of Laennec's cir-

* In the biopsies which were done subsequent to this series, two accidents occurred. In one, bile was aspirated from the gallbladder which, as shown by subsequent autopsy, had been punctured by transfixing the anterior part of the liver. This was uncomplicated and had nothing to do with the patient's death. In the other, the duodenum was apparently penetrated, as the biopsy consisted of duodenal mucosa. There was no subsequent fever, tenderness, or disability. Such incidents, however, concretely illustrate the danger of the procedure and emphasize the care which should be taken with each attempt.

TABLE 2. PATHOLOGIC DIAGNOSES FROM BIOPSY SPECIMENS BEFORE AND AFTER INTRODUCTION OF SPECIAL NEEDLES

Diagnosis	1933-35	1943-45	Total
Carcinoma (all types)	8	13	21
Carcinoma with Laennec's cirrhosis	0	5	5
Fatty metamorphosis	3	7	10
Laennec's cirrhosis	1	6	7
Biliary stasis	0	5	5
Hemosiderosis	0	3	3
Neurosar degeneration	1	2	3
Acute hepatitis	0	3	3
Acute cholangitis	0	1	1
Fibrosis	0	1	1
Amorphous	0	1	1
No pathologic diagnosis	2	1	3
Unsatisfactory	10	5	15

TABLE 3. CORRELATION OF AUTOPSY AND BIOPSY DIAGNOSES

Biopsy diagnosis	Autopsy diagnosis
1933-1943	
1. Blood in hepatic	Well-differentiated squamous cell carcinoma of esophagus with metastases
2. Tumor, liver and blood	Primary carcinoma of liver
3. Liver—no pathologic diagnosis	Focal fibrosis of liver
4. Partially differentiated adenocarcinoma	Partially differentiated squamous cell carcinoma of bronchus
5. Slight fatty metamorphosis; acini of columnar epithelium	Well-differentiated adenocarcinoma of liver—bile duct type
6. Carcinoma—type undetermined	Undifferentiated carcinoma of trachea with metastases
7. Laennec's cirrhosis	Laennec's cirrhosis
8. Normal glycogen storage	Fatty metamorphosis
1943-1945	
1. Hemosiderosis, slight chronic periportal hepatitis	Hemochromatosis; Laennec's cirrhosis
2. Partially differentiated adenocarcinoma metastatic to liver	Partially differentiated adenocarcinoma of body of pancreas
3. Undifferentiated carcinoma of liver	Undifferentiated carcinoma of the bronchus with metastases
4. Liver—no pathologic diagnosis	Passive hyperemia of liver
5. Fatty metamorphosis, marked; fibrosis consistent with clinical diagnosis of Laennec's cirrhosis	Probable primary carcinoma of liver; Laennec's cirrhosis
6. Focal fibrosis—consistent with Laennec's cirrhosis	Laennec's cirrhosis
7. Consistent with Laennec's cirrhosis	Laennec's cirrhosis
8. Secondary carcinoma of liver—probably squamous cell	Partially differentiated squamous cell carcinoma of esophagus with metastases
9. Laennec's cirrhosis, fatty metamorphosis	Laennec's cirrhosis
10. Undifferentiated carcinoma, fibrosis and atrophy, consistent with primary carcinoma of liver	Primary carcinoma of liver; Laennec's cirrhosis

rhosis was made when a sufficiently large sample was obtained to rule out subcapsular or focal fibrosis. The classification of acute hepatitis includes both periportal and diffuse types. The "unsatisfactory" cases are those in which sufficient tissue was lacking for a histologic diagnosis. Of the 5 unsatisfactory instances in the later group, 3 were in patients who subsequently had satisfactory biopsies.

The autopsied cases (Table 3) afford an opportunity to check the accuracy of the biopsy diagnoses. In the period 1939-1943, 4 may be considered to be correct, whereas in 2—bile duct carcinoma (No. 5) and focal fibrosis (No. 3)—the lesion was missed. The case of carcinoma of the esophagus metastatic to the liver (No. 1), in which only blood was obtained by biopsy, must be considered a gross error. The diagnosis of normal glycogen storage was undoubtedly correct but was not subject to check at autopsy because of post-mortem tissue changes.

During the period from July 1, 1943, to 1945, 7 of the 10 biopsies may be considered correct and in 1, a carcinoma (No. 5), probably primary in the liver, was missed although the accompanying cirrhosis was correctly diagnosed. In the case of passive hyperemia diagnosed at autopsy (No. 4) there is question as to whether this change was present when the biopsy was taken. The biopsy diagnosis of hemosiderosis and slight chronic periportal hepatitis (No. 1) is not altogether satisfactory in the case of hemochromatosis, although it is consistent.

To assess the clinical value of these biopsies is difficult and at least in part subjective. When the charts were reviewed, the biopsies were considered worthwhile if they established a primary diagnosis or confirmed a clinical impression. If they were not in agreement with the general clinical picture but could not establish a diagnosis or were unsatisfactory biopsies, they were considered not helpful. In the early group, 13 of 26 attempted biopsies were helpful (50%). However, 13 of 16 successful biopsies were

helpful (80%). In the latter period, 38 biopsies were helpful clinically. These constitute 71.6% of the total 53 cases or 79.2% of the 48 successful cases.

Of the 68 livers from which biopsies were taken, 31 were described as nodular or irregular, and of these 18 (58%) were diagnosed carcinoma by biopsy. Of the total, only 4 livers were not palpable and in 3 of these biopsy was successful.

Indications and uses of liver biopsy are somewhat limited but nevertheless important. It is felt as a result of experience gained in this series of cases that any case of hepatomegaly in which the etiology is questionable deserves a biopsy. Biopsies may be used in following the progress and evaluating the therapy of chronic hepatic disease such as cirrhosis. Investigation of the parenchymal changes in epidemic hepatitis,^{3,4} toxic hepatitis, Weil's disease and other conditions may be greatly benefited by frequent biopsies during the course of the illness. Vital studies of living tissue obtained by human liver biopsy may aid materially in elucidating the normal physiologic function of the liver and its alterations in metabolic diseases. Generally, it is felt that only easily palpable livers should be needed. Although routine bleeding, clotting, and prothrombin times were not done in these cases, it is believed that they should be as an added precaution.

The intercostal approach advocated by some authors is not recommended. There is danger of tearing the liver should the patient inadvertently breathe while the needle is in the liver and held rigidly between the ribs. Acutely ill patients cannot cooperate well enough to permit the procedure, and needless transfixion of the pleural space may result in empyema, traumatic pneumothorax, or air embolism.

The use of peritoneoscopy in every case is not recommended. The procedure requires expert training and an expensive instrument and is therefore not widely available. In addition, in many cases it adds little to the biopsy procedure. In those cases in which the liver is not palp-

able or in which a nodule is suspected but cannot be felt peritoneoscopy may be valuable in guiding the biopsy needle. However, it is felt that routine peritoneoscopy adds needle-ly to the risk in each case.

The 2 needles in current use at Cleveland City Hospital each have their advantages. The Silvermann instrument is smaller and yields smaller specimens, but it is easier to handle. The sections made from these specimens show a moderate amount of tissue distortion due to pressure. The Roth-Turkel needle is larger, yields larger specimens, and produces little or no distortion of tissue. It is a little more difficult to control than the smaller needle.

From the pathologist's standpoint needle biopsy of the liver has certain advantages and certain limitations. Cytology in a properly obtained specimen is usually superior to that obtained at autopsy for the specimen is obtained from the living organism and fixed with a minimum of autolysis. Studies of glycogen content, for example, are more exact than those of autopsy specimens. However, cytology may be very poor in those instances where there has been distortion by pressure or shredding. A second advantage of the procedure is that it permits observation at intervals during life. This offers information as to the pathogenesis of certain diseases and the effects of treatment. For example, in Case 2 repeated biopsies of a liver the seat of Laennec's cirrhosis showed, in the first instance, marked fatty metamorphosis and in the second none. This may be accepted as evidence in discrediting the classification of fatty cirrhosis as an entity separate from Laennec's cirrhosis.

The limitations of the procedure are those relating to the examination of limited material. Lesions may be focal and missed by random sampling. Furthermore, certain diseases by definition imply a diffuse change. For example, if a biopsy shows perilobular fibrosis, bile duct regeneration and alteration of lobular archi-

ture, a diagnosis of Laennec's cirrhosis is made. Actually, it should be stated (as we have stated in some instances) that if the biopsy is representative of the entire liver, the diagnosis is Laennec's cirrhosis. Focal areas of atrophy and fibrosis can imitate the histology of Laennec's cirrhosis. This is particularly true of the so-called "capsular pseudocirrhosis."

In some instances the material offered for examination is limited, including only a portion of a lobule. In these instances a complete objective diagnosis may be unwarranted but it is still possible to contribute information which, taken with other clinical data, may aid in diagnosis. For example, in the case of hemochromatosis, discussed earlier in this paper (Table 3), the objective diagnosis of cirrhosis was not thought to be justified due to the limited material. The hemosiderosis (Fig. 8), however, was of the type and of the degree seen in hemochromatosis.

The diagnosis of tumors, likewise, has the limitations of inadequate material. This is especially true of the diagnosis of primary carcinoma of the liver. However, since the introduction of the Roth-Turkel needle, generous samples have been submitted and the histologic picture in many instances could not be greatly improved on by formal surgical biopsy.

Several cases are cited briefly to illustrate the use of liver biopsy.

Report of Cases. CASE 1. F.S., a 63 year old white man in whom a diagnosis of cirrhosis of the liver was made by surgical biopsy during laparotomy in 1943, was admitted to the medical service of the Cleveland Clinic Hospital May 2, 1945, complaining of swelling of the abdomen and ankles, weakness and shortness of breath of 2 months duration. Examination revealed a distended abdomen with dilated superficial veins. A fluid wave and shifting dullness were present. The liver, which was enlarged to 5 cm. below the costal margin, was quite hard. There was 1 nodule about 4 or 5 cm. in diameter palpable at the edge. Biopsy (Fig. 2) done on May 3 revealed "Laennec's

cirrhosis, slight periportal hepatitis." Repeat biopsy on July 13 showed little change.

CASE 2.—F. Z., a 29 year old white man was admitted to the medical service March 8, 1945, complaining of pain and swelling in the abdomen, malaise and fever. Examination revealed a distended, tender abdomen with a large, firm liver felt at the level of the umbilicus. Biopsy (Fig. 3) March 12 revealed Laennec's cirrhosis with fatty

empyema for many years. Examination revealed a smooth, firm, markedly enlarged liver. Biopsy (Fig. 5), June 19, was reported "moderate amyloidosis of liver."

CASE 4.—J. S., a 59 year old white man was admitted to the medical service July 16, 1945, with indefinite history of abdominal pain for 2 months and a weight loss of 30 pounds in the past year. There were signs of atelectasis in the left upper lung

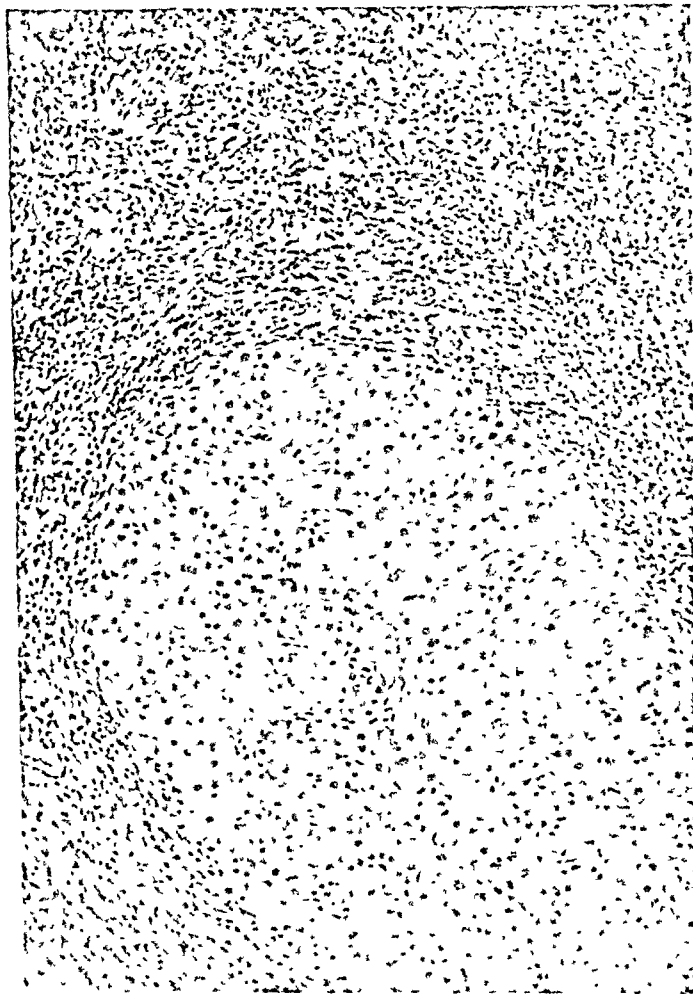


FIG. 2.—(S 33281) L. P. Advanced Laennec's cirrhosis—note lobule surrounded by fibrous tissue which contains many new bile ducts and shows slight round cell infiltration.

metamorphosis and acute hepatitis. After symptomatic and supportive therapy, repeat biopsy (Fig. 4) on May 16 showed disappearance of the fatty metamorphosis and acute hepatitis. The patient was clinically much improved and the liver was much smaller than previously.

CASE 3. C. G., a 59 year old white man was admitted to the surgical service May 31, 1945, with a history of chronic draining

field and several firm, 1 to 2 cm. cervical lymph nodes. The liver, which was 10 cm. below the right costal margin, was hard, nodular and tender. Biopsy (Fig. 6) on July 24 showed undifferentiated carcinoma of the liver of unknown origin. Subsequently autopsy revealed an undifferentiated carcinoma of the upper lobe of the left lung and widespread metastases to the liver and cervical and mediastinal lymph nodes (Fig. 7).

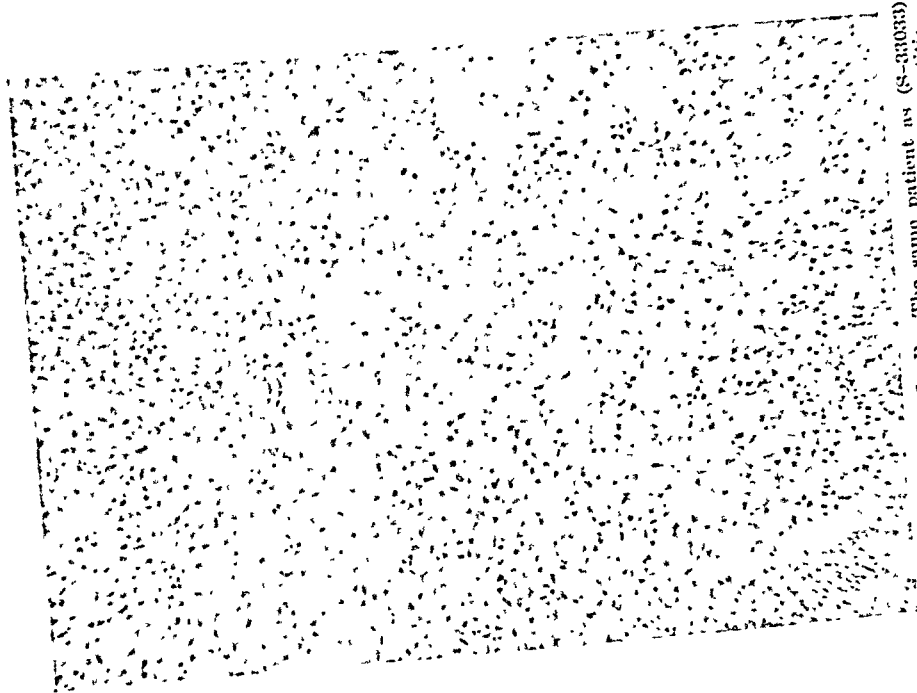


FIG. 4.—(S-33300) L. P. The same patient as (S-33033) showing disappearance of fatty changes and acute hepatitis.

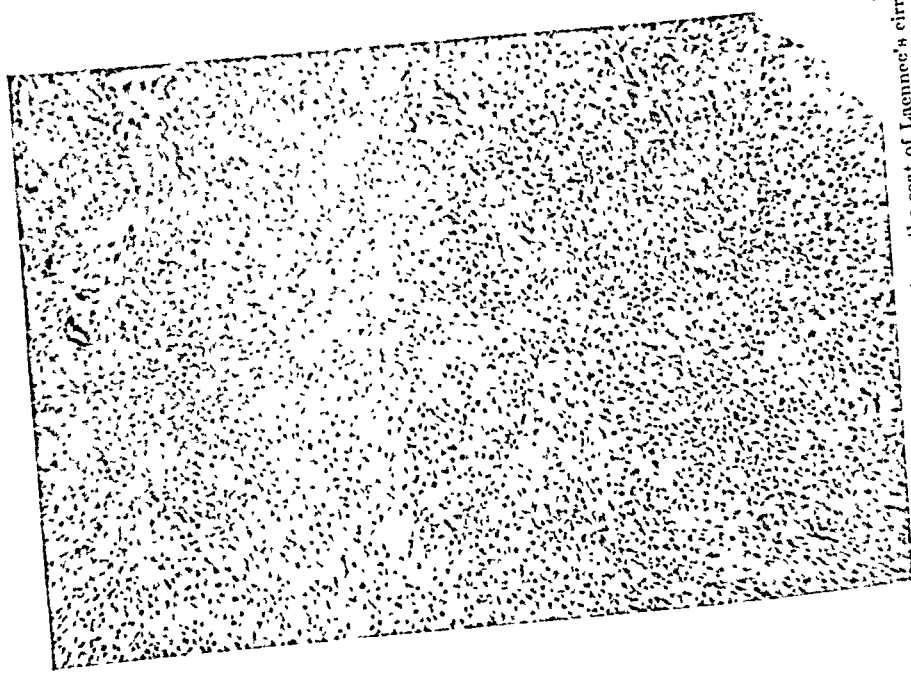
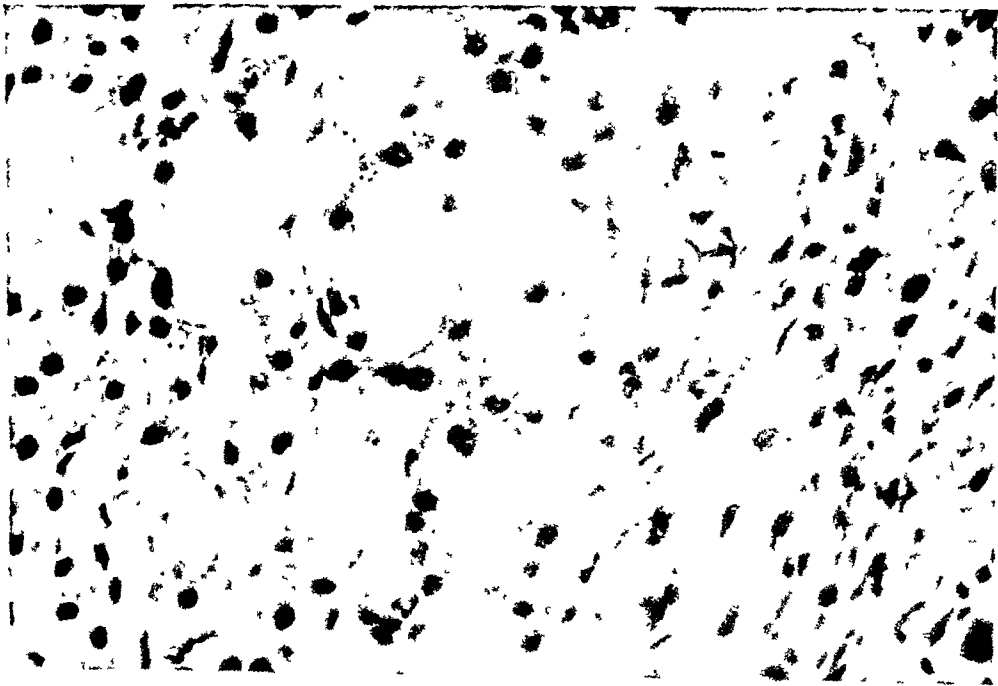


FIG. 3.—(S-33033) L. P. Liver, the seat of Laennec's cirrhosis showing also acute hepatitis and marked fatty metamorphosis.



B



A

FIG. 5.--(S 33516) L. P. and H. P. A and B, Amyloidosis of liver.

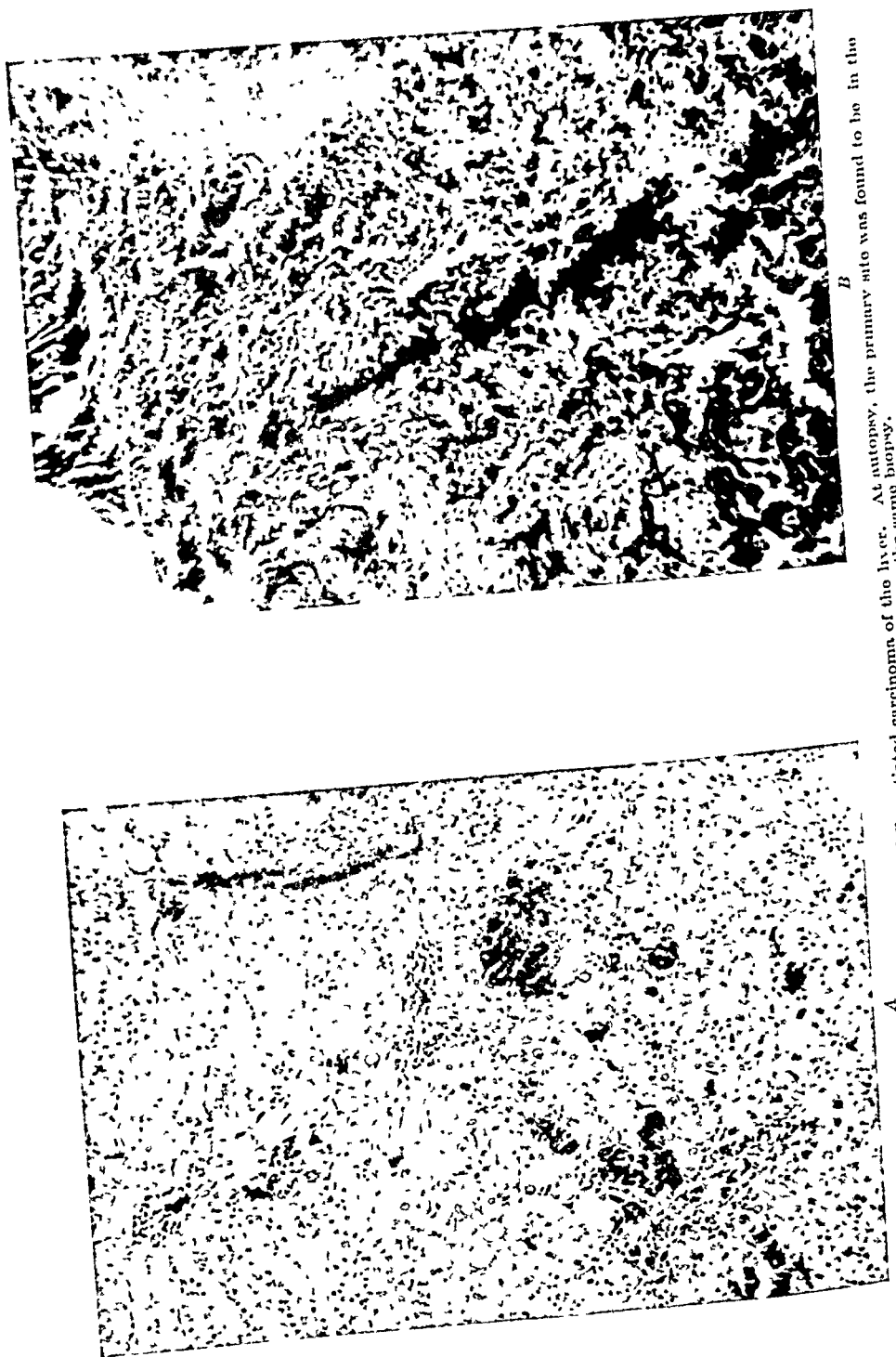
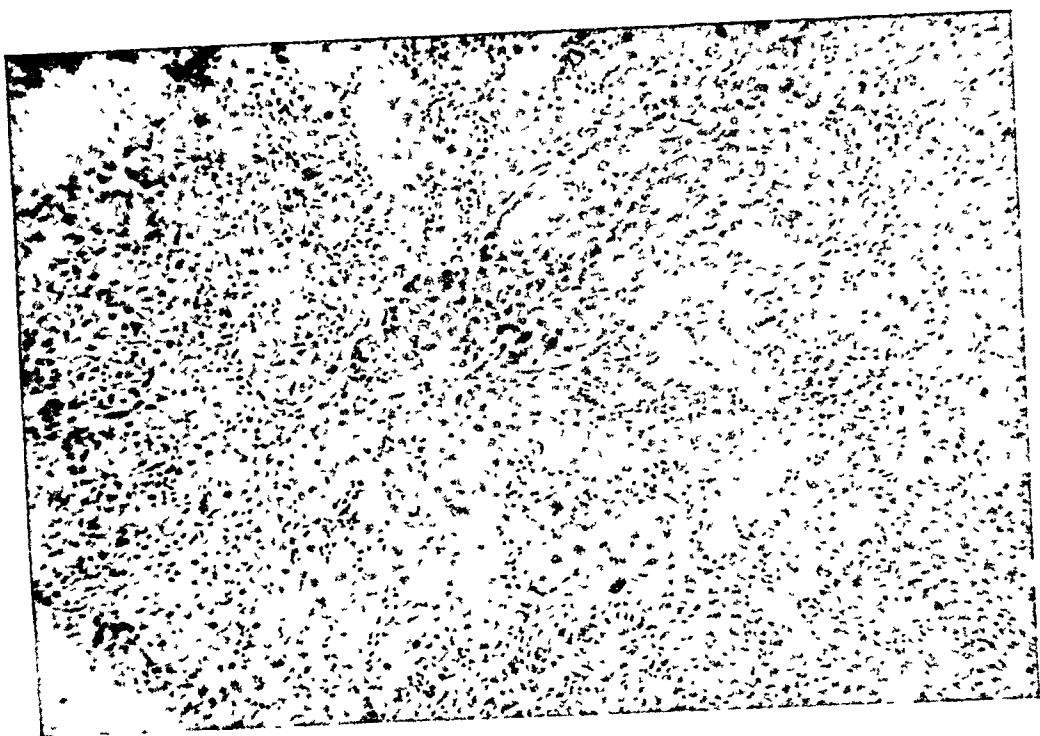
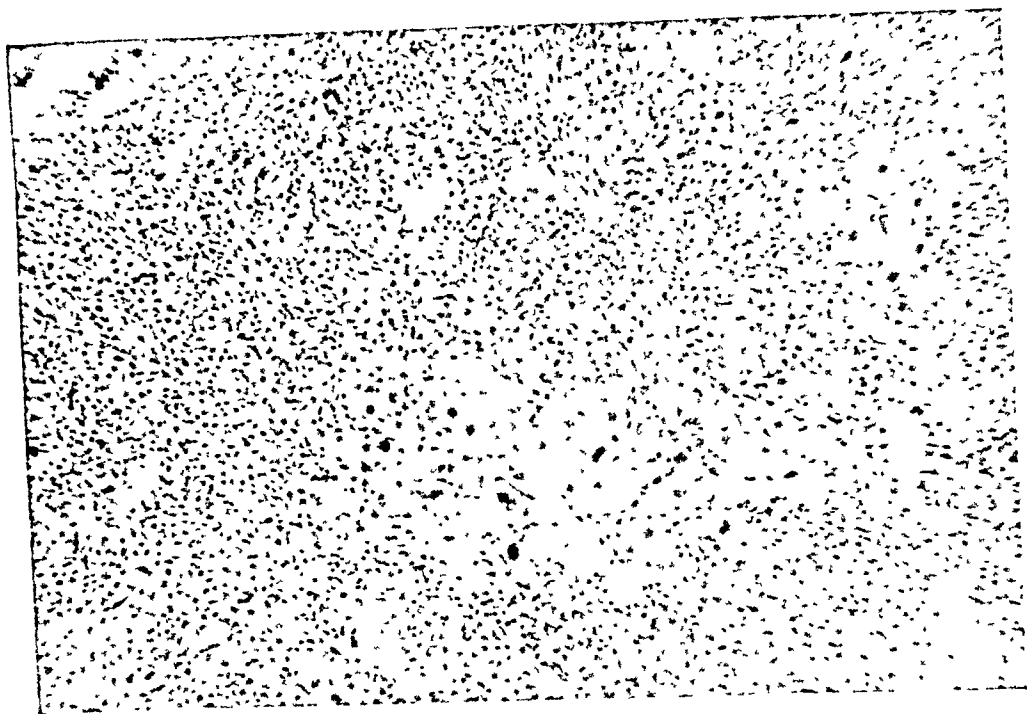


FIG. 6.—(S-3367) L. P. A, Metastatic undifferentiated carcinoma of the liver. At autopsy, the primary site was found to be in the left bronchus. B, Another area from the same biopsy.



B



A

FIG. 7.—(S-33580.)—A. and B. Two areas of primary carcinoma of the liver with atrophy and fibrosis.

CASE 5.- E. V., a 59 year old white man, was admitted to the medical service, July 5, 1915, complaining of pain in the abdomen of 1 month's duration followed in 1 week by appearance of a mass. He had lost 25 pounds. Examination revealed a hard, nodular liver extending 10 cm. below the costal margin. Biopsy (Fig. 7), July 6, was reported undifferentiated carcinoma of the liver with atrophy and fibrosis consistent with primary carcinoma of the liver. Necropsy showed nodular, primary carcinoma of the liver.

Summary and Conclusions. Needle biopsies of the liver have been done on 68 patients in Cleveland City Hospital since 1939. Biopsies have been most useful in the diagnosis of cancer of the liver and in establishing the nature of diffuse parenchymal liver disease including cirrhosis. The occasional fatalities reported by others have so far not been encountered in this series.

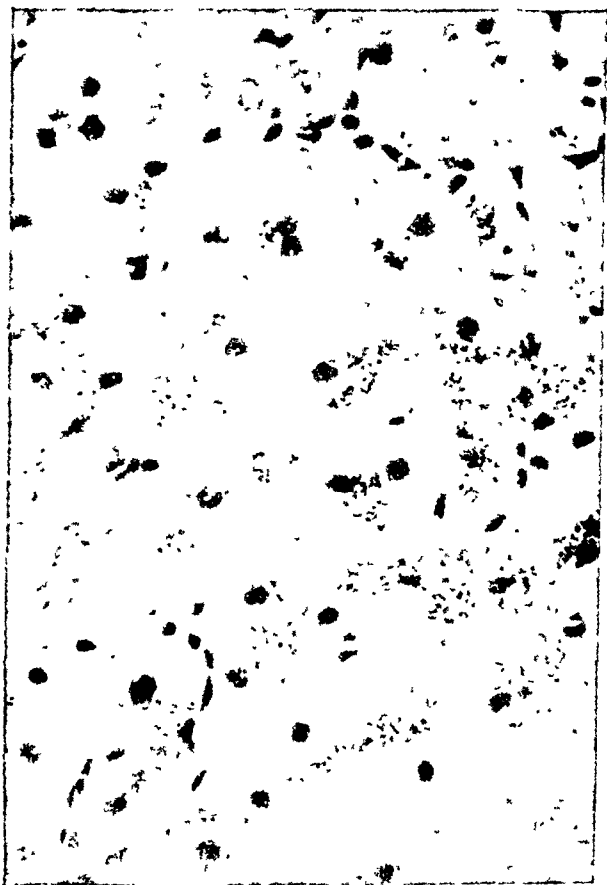


FIG. 8.—(S-32371.) Hem siderosis of liver from a case of hemochromatosis.

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PLASMA PROTEINS

I. ALTERATION IN DIABETIC RETINITIS

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The purpose of this paper is to report studies made on 31 patients with diabetic retinitis. The work was done chiefly to determine any correlation between certain alterations in the plasma proteins and retinal changes and to determine whether a high protein intake would alter either or both of these disorders.

Previous studies³ have shown that the plasma protein pattern (Tiselius technique) is frequently abnormal in diabetes mellitus when the condition is inadequately treated. The typical change found in the protein fractions was a reduced level of albumin and an increase in β globulin. The total quantity of protein may be normal or only slightly reduced. In such cases the levels of the plasma proteins can be restored to normal by adequate treatment of the diabetes if complications such as infection, renal insufficiency or retinitis are not present. In cases of diabetic retinitis restoration of the plasma protein levels to normal was brought about with much more difficulty. Higher protein diets were required and the change was much slower. In instances in which renal disease and albuminuria were present in addition to the retinitis, the plasma protein abnormalities were not always corrected by the methods employed.

The term, "adequate treatment," as used in this paper, means a careful attempt to maintain the patient in normal strength and at or near ideal weight, to maintain as far as possible the urine free from sugar at all times, normal blood sugar levels both fasting and 4 hours after meals, and a normal total blood cholesterol level.

Methods. Longsworth's modification⁴ of the Tiselius electrophoretic method was used to estimate the albumin, α globulin, β globulin, γ globulin, and fibrinogen in the plasma. Phosphate buffer solution of pH 7.8, ionic strength 0.16 was employed. Normal values determined in this laboratory on 21 subjects have been reported.³ A more complete description of the method as employed is given in our previous paper.¹ Total protein values were determined by Pregl's modification of the micro-Kjeldahl method for estimating total and non-protein nitrogen.

Urea clearance tests of kidney function were done by the technique of Moller, McIntosh and Van Slyke.⁶

Results. A number of factors were used to evaluate these 31 cases of diabetic retinitis. These factors included sex and age distribution, duration of the diabetes, presence or absence of proteinuria, of hypertension and of reduced urea clearance values, adequacy of treatment, total duration of such treatment, protein intake, electrophoretic analysis of plasma proteins and progress of the retinal changes.

An analysis of the sex distribution reveals that diabetic retinitis is more common in women than in men. Of the total of 31 patients, 26 (84%) occurred in women and 5 (16%) in men (Table 1). The high incidence of diabetic retinitis in women also characterized Bedell's series.⁵ This is in contrast to the almost equal distribution of diabetes mellitus in the 2 sexes.

Age is also an important factor; 25 patients (80%) were between 40 and 70 years of age (average 51) (Table 1).

The average duration of the diabetes

for both sexes as judged by the history was 11 years. In contrast, the symptoms assumed to designate the onset of retinitis were of relatively recent origin. Diabetic retinitis in men was not recognized until diabetes had been present from 12 to 25 years (average 19) and in women until diabetes had been present for 2.5 to 22 years (average 10).

Proteinuria was common when the diabetes was untreated but often disappeared during adequate management. A trace of albumin was observed in 13 patients initially, but disappeared during management in 5 of the 11 who had subsequent urinalyses.

occasionally at intervals of 2 or 3 months. The urinalyses made at home seldom showed glycosuria. Each reëxamination of the patient included blood and urine sugar determinations fasting and 3 or 4 hours postprandially at noon and occasionally 3 or 4 hours postprandially in the afternoon. An effort was made to maintain blood sugar levels at normal levels, and only occasionally was the attempt unsuccessful. In a few instances isolated blood sugar levels were as much as 40 mg. % above normal. Normal levels are taken to be 80 to 120 mg. per 100 ml. of venous blood, Myers-Bailey method.

TABLE 1.—AGE AND SEX DISTRIBUTION IN CASES OF DIABETIC RETINITIS STUDIED

Age (yrs.)	Men	Women	Total
20-29	1	1	2
30-39	1	0	1
40-49	1	5	6
50-59	0	12	12
60-69	2	5	7
70-79	0	3	3
Total	5	26	31

Hypertension was present in approximately half of the group; 17 (55%) had a systolic blood pressure of 150 mm. of mercury or more; 13 (42%) had a diastolic blood pressure of 90 mm. of mercury or more.

Urea clearance tests were done in 9 patients. In 5, clearance values averaged 80 % or more, and of these all but 1 showed persistent proteinuria. The remaining 4 patients had urea clearance values of 20, 30, 36 and 64% respectively. The blood urea was elevated above 55 mg. per 100 ml. in 3 patients who did not have urea clearance determinations.

Adequate treatment for a number of months was obtained in 10 patients who will be discussed in greater detail. These patients were specially selected as individuals who maintained excellent diabetic control throughout the period of study. Each patient made urinalyses at home 3 or 4 times daily and had blood sugar levels determined at monthly intervals or

In this group of patients the protein intake was varied from time to time and the effects were correlated with shifts in their plasma protein patterns.

Total Plasma Proteins. We have found the normal total plasma protein⁴ to range from 5.9 to 7.8 gm. per 100 ml. with an average of 6.5 gm. The initial total protein was within this range or only slightly below in 29 patients. The remaining patients had advanced renal disease. The initial total protein values in these 2 instances were 4.8 and 5.3 gm. per 100 ml.

Albumin Fraction. The normal range for albumin fraction⁴ was 3.7 to 5.1 gm. per 100 ml. with an average value of 4.1 gm. The plasma albumin level was low in 28 (90%) of the 31 patients. The individual determinations ranged from 2.06 to 3.8 gm. per 100 ml. with an average of 3.2 gm. The lowest values were obtained in 4 patients with impaired renal function. The 3 remaining patients with normal albumin levels were all under ade-

quite management from 3 to 19 months before the first determinations were conducted.

β Globulin Fraction. The normal range of β globulin determined in 25 normal controls in our laboratory⁴ were 0.65 to 1.07 gm. per 100 ml.; the average was 0.81 gm. Of the 31 patients, 29 (93%): demonstrated an elevated β globulin fraction; the average was 1.29 gm. per 100 ml.

Let us now consider the effects of variation in protein intakes upon the plasma protein level in the 10 carefully selected patients who can be considered as having received adequate treatment. Plasma proteins were subjected to electrophoretic analysis repeatedly in these 10 patients. Four of the 10 received relatively low daily protein intakes. One of the 4 with a low albumin level initially maintained an intake of 50 gm. protein per day for 10 months. A further fall in plasma albumin level occurred. The plasma albumin of another patient increased to nearly normal levels while receiving 80 gm. protein per day for 3 months. The remaining 2 patients, who had received 77 and 84 gm. of protein per day for 10 days and 5 months respectively, showed no alteration in plasma albumin levels.

The other 6 of this group were given a much larger daily protein intake, chiefly as meat, for a period ranging from 3 weeks to 6 months. Four of the 6 had a significant elevation in albumin fraction at the end of the test period. One of these 4 had a normal albumin level 3 months after the protein intake was increased from 70 to 140 gm. per day. Two others showed a significant rise in this same fraction 3 weeks and 6 weeks after protein intakes were elevated by 40 gm. per day to 120 and 140 gm. respectively. The albumin fraction in the fourth patient gradually returned to normal 6 months after the protein intake was increased from 76 to 136 gm. per day.

In the remaining 2 patients given a high protein intake, the albumin levels failed to increase over a period of 3 months and 1 year respectively. The protein in the

diet was raised from 60 to 140 gm. daily in 1 and from 60 to 120 gm. per day in the other. Persistent proteinuria was present in each patient, but urea clearance values were not obtained.

No case was seen demonstrating a fall in plasma albumin when more protein was prescribed in the diet. In most instances where albumin levels became normal, β globulin levels approached normal.

The 4 patients who were regarded as improved showed the following course of events: the first patient entered the period of study with concentric contraction of both form and color fields in the right eye and vision was 6/12-1. There was loss of the inferior half and partial loss of the temporal and nasal superior form fields in the left eye. In addition, concentric contraction of color fields was observed. Vision was 6/30-1. One year later central fields were normal. Ophthalmoscopic examination on 5 occasions showed no retinal hemorrhages (Case 1, Table 2).

The second patient originally had a loss of green color field and a relative central scotoma for blue in both eyes. Several months later the green field was normal and the central scotoma had disappeared. Two small scotomas in the central field of the right eye persisted. The left central field was normal when first tested and remained unchanged. No new hemorrhages appeared. Visual acuity measured 6/9-1 in both eyes at the beginning and the end of the period studied (Case 2, Table 2).

There were no new hemorrhages in the 2 remaining patients during a 6 month and 2 year period of observation. Subjectively, vision was improved, but no actual measurement was obtained.

Discussion. Patients with uncomplicated diabetes mellitus may have low plasma albumin levels when untreated or poorly treated. Adequate treatment of the diabetes was almost invariably accompanied by a prompt return to normal of the albumin level. The diet needed

only to contain 1 gm. of protein per kg. body weight.

Patients with uncontrolled diabetes accompanied by retinitis had much lower plasma albumin levels and higher β -globulin levels than were observed in the uncomplicated cases. With adequate treatment of the diabetes the protein levels were improved but not restored to normal unless the diet contained a high daily protein intake. Proteinuria apparently accentuated the hypoproteinemia and the albumin fraction was not restored to normal by increasing the protein in the diet.

value of high protein intake in cases of diabetic retinitis can be drawn.

Summary. 1. Plasma proteins in patients with diabetic retinitis are characterized by low albumin, high β globulin and a tendency toward normal total protein values.

2. Adequate management of the diabetes when diets contained a maintenance amount of protein improved but did not correct the plasma protein pattern in patients having retinitis.

3. When adequate treatment was instituted and the daily protein intake increased to 120 to 140 gm., the plasma protein

TABLE 2.—TISELIUS PROTEIN FRACTIONATION ON 2 PATIENTS—CHANGES OBSERVED ON REPEATED STUDIES AFTER INCREASED PROTEIN INTAKE

Case	Diet			Observation period		Date	Total protein	Alb.	α Glob.	β Glob.	γ Glob.	Fibrinogen
	C	P	F	From	To							
1	130	56	130	2/ 3/43	11/30/43	10/ 8/43	6.12	3.37	0.36	1.37	1.02	
						11/30/43	6.28	2.85	0.39	0.64	0.77	0.63
	130	80	120	11/30/43	12/20/43	12/20/43	6.42	4.03	0.53	0.76	0.61	0.49
2	100	71	50	3/15/44	6/16/44	6/16/44	6.54	3.43	0.38	1.26	1.12	0.38
	100	100	50	6/22/44	8/ 1/44	8/ 1/44	6.70	3.56	0.30	1.37	1.20	0.36
	100	140	50	8/ 1/44	9/25/44	9/25/44	7.10	3.99	0.28	0.92	1.24	0.57

Both patients showed: (1) gradual rise in total protein; (2) gradual rise in total albumin; (3) gradual fall in total β globulin.

During the interim of study visual fields improved, visual acuity improved, and the retina showed fewer hemorrhages. Neither patient had albuminuria or measurable impairment of renal function.

The greatest improvement in retinal changes occurred in those patients in whom high protein intake restored the plasma protein pattern to normal. This observation suggests the value of diabetic diets of high protein content as an adjunct in the prevention or treatment of diabetic retinitis. It is evident, however, that a much larger series must be studied over a longer period of time before final conclusions regarding the therapeutic

pattern either improved or returned to normal, provided serious renal disease was not present.

4. The 4 patients in whom high protein diets restored the plasma proteins to normal showed the greatest retinal improvement.

5. From these studies, it appears that a high protein diet may be a useful adjunct in the management of diabetic retinitis.

We wish to express our sincere appreciation to Dr. A. D. Ruedemann and Dr. Jacob Moses for their cooperation in following the retinal changes and to Mr. James Clark for performing many of the analyses.

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THE FALLACY OF THE EXTON-ROSE GLUCOSE TOLERANCE TEST

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It is the purpose of this paper to present information from the literature which indicates that the Exton-Rose 2 dose, 1 hour, glucose tolerance test is based upon a physiologic misconception. In addition, the results of a series of well-controlled glucose tolerance tests are presented to support further this contention.

In a previous paper ¹ of us⁹ reviewed the pertinent literature and pointed out certain discrepancies which indicated that the Exton-Rose test was less reliable than the conventional 1 dose test. Since 1942, 2 additional studies^{16,21} on the Exton-Rose test are worthy of note and indicate that this test is still considered a worthwhile procedure. The use of 2 doses of glucose, one initially and the second $\frac{1}{2}$ hour later, as employed in the Exton-Rose test⁵ was inspired by the paradoxical law of Allen¹ which states that the more sugar given to a normal individual, the more it is utilized and that the limits of tolerance in normal individuals are apparent rather than real. Therefore, there is no real limit to the ability of a normal person to utilize glucose. The above is exemplified by the Hamman-Hirschman effect⁸ in which the resultant peaks in the blood sugar curves become progressively lower after successive doses of glucose. Exton and Rose⁶ believed that if 50 gm. of glucose were given orally and then followed in $\frac{1}{2}$ hour by a similar dose a paradoxical result would occur in that the blood sugar would either fall in the second $\frac{1}{2}$ hour of the test or at most fail to rise appreciably. In arriving at the conclusion that a second 50 gm. dose of glucose would have any

appreciable effect upon the blood sugar curve in the short space of $\frac{1}{2}$ hour, Exton and Rose failed to take into consideration certain physiologic facts, namely: (1) the rate of absorption of glucose from the gastro-intestinal tract, (2) its rate of utilization, and (3) the natural shape of a glucose tolerance curve after giving 100 gm. of glucose, whether it is given in 1 dose or in divided doses of 50 gm., at $\frac{1}{2}$ hour intervals. Let us consider each of these factors in turn.

With regard to intestinal absorption, it has been estimated by Cori and Cori,⁴ after a comprehensive review of the literature, that the maximum rate of absorption from the gastro-intestinal tract in man is 0.85 gm. per kilo, or about 60 gm. per hour for an average sized man. Actual measurements of absorption by Warren, Karr, Hoffman and Abbott²⁰ in man indicate that the maximum rate of absorption is 43 gm. per hour and usually less than this. Thus, the Exton-Rose test supplies twice as much glucose as the gastro-intestinal tract is capable of absorbing in 1 hour. In other words, it adds 50 gm. of glucose to the stomach while there is still an excess in the upper intestinal tract. Groen⁷ concludes that the absorption of a glucose test meal of 50 gm. would be 4 hours or somewhat less. We think that this is too conservative. Groen believes that if the glucose were introduced directly into the whole intestine a greater absorption would occur, but that when glucose is given by mouth the pylorus takes care that absorption does not take place at a rate which

exceeds the capacity of the storage mechanism. The latter portion of this statement is in keeping with the result of Ravdin, Johnston and Morrison,¹⁴ who found that when glucose solutions of varying concentrations from 3.5 to 50 % are placed in the stomach, the concentrations obtained from the jejunum and ileum at the end of 1 hour are remarkably constant and did not exceed 5.3%. Thus, the stomach protects the intestine from receiving too much glucose in too great concentrations.

The following pertinent excerpt is quoted from Peter's and Van Slyke's "Quantitative Clinical Chemistry:"¹³ "Apparently the mechanism for the utilization of sugar by the tissues is not greatly accelerated until the blood sugar reaches a certain height. The activity of the mechanism increases as the blood sugar rises higher, until, at the peak of the curve, utilization rate overtakes, and then surpasses the absorption rate, in spite of the fact that the absorption may still remain at its maximum. The accelerated utilization, once speeded up, does not stop until the blood sugar has become subnormal. As shown by Cori, absorption from the intestine cannot surpass a certain maximum rate; if more glucose is ingested beyond the amount eliciting complete absorption activity, absorption continues for a longer time, but not at a faster rate. Consequently, when glucose utilization is stimulated to outrun this maximum inflow, the blood sugar begins to fall from its peak, and continues to do so even though the intestine is still loaded with glucose."

In view of the above passage it would seem pointless to give a second dose of glucose at the height of the blood sugar curve, since in a normal individual the curve would fall whether a second dose were given or not given. In a diabetic the curve would continue to rise whether 1 initial dose or 2 doses at $\frac{1}{2}$ hour intervals were given. Therefore, any difference in the effect which the administration of 1 dose of 100 gm. or 2 doses of 50 gm.

each would have upon the blood sugar curve would not be manifested during the 1st hour or the duration of the entire Exton-Rose test.

Relative to the maximum rate of glucose utilization, experience has shown that 25 to 30 gm. of glucose is optimal for the 1 hour intravenous tolerance test.^{11,19} This direct introduction of a sizable quantity of glucose into the blood stream provides a much more intense stimulus to the glucose disposal mechanism than the slow absorption after oral administration. The fact that the body requires 1 hour to dispose of 25 gm. of glucose given intravenously, thereby producing the maximum stimulus, makes it seem unlikely that the disposal of an oral dose of 50 gm. could occur in $\frac{1}{2}$ hour and any appreciable response be manifested to a second dose of 50 gm.

The final factor concerns the natural shape of the oral glucose tolerance curve. It is well known that in the glucose tolerance test in normal people the peak of the curve, depending on absorption and utilization, will usually occur between 30 and 60 minutes. If absorption and utilization of glucose are such that the curve rises slowly, the peak will probably occur at 60 minutes. On the other hand, if absorption is more rapid and utilization less so during the first 30 minutes, the peak will occur more abruptly, the glucose disposal mechanism will be sharply stimulated, and the curve will fall during the second $\frac{1}{2}$ hour. We believe these phenomena will occur regardless of whether the glucose is given in 1 dose of 100 gm. or in divided doses of 50 gm., as demonstrated in our series of parallel glucose tolerance tests in the same individuals shown in Table 1.

Method. Ambulatory patients on the medical service were used for this study. These patients were afebrile, with mild non-disabling diseases, and had been prepared by generous antecedent carbohydrate diet since the importance of the last-mentioned factor has been repeatedly demonstrated.^{3,10,12,15} A 12 hour rest and fast pre-

ceded each test. The tests were done within 2 to 5 days of each other. The general condition, activities and emotional status of the patient were observed and it is believed that these were practically constant for several days prior to the performance of each pair of tests in a given individual. Venous bloods were taken at exact times by 1 of 2 individuals highly skilled in venepuncture with minimum use of a tourniquet and the blood sugar was determined in duplicate on each specimen according to the method of Folin and Wu using a photoelectric colorim-

column of Table 1 showing the difference between the 30 and 60 minute blood sugar values. Table 2 shows that in the 1 dose test 3 cases had an average rise of 6 mg. per 100 cc., whereas 7 cases had an average fall of 20 mg. per 100 cc.; while in the 2 dose test 5 cases had an average rise of 12 mg. per 100 cc. and 5 cases had an average fall of 13 mg. per 100 cc. Thus it is obvious that the blood sugar curve in the 2 dose test of Exton and Rose does not fall in the second $\frac{1}{2}$ hour

TABLE 1.—A COMPARISON OF THE RESULTS IN THE 1 DOSE GLUCOSE TOLERANCE TEST AND THE EXTON-ROSE TEST IN THE SAME INDIVIDUALS

(In each instance, the 1 dose test is listed first)

Case No.	Days between tests	Fasting (mg./100 cc.)	30 min. (mg./100 cc.)	60 min. (mg./100 cc.)	90 min. (mg./100 cc.)	120 min. (mg./100 cc.)	Differences between 2nd and 3rd specimen
1	2	98	135	130	99	93	+ 4
		88	122	144	140	94	+22
2	2	78	121	97	102	80	-27
		97	107	119	93	98	+12
3	5	76	123	110	105	59	-13
		78	118	110	86	79	- 8
4	2	83	120	108	88	79	-12
		87	101	93	76	98	- 8
5	2	90	128	136	122	96	+ 8
		91	142	127	100	92	-15
6	2	81	130	100	99	90	-30
		78	121	110	87	85	-11
7	2	78	140	112	90	86	-28
		74	144	146	96	79	+ 2
8	2	79	128	116	85	98	-12
		80	122	143	92	103	+21
9	4	74	114	119	104	90	+ 5
		80	110	115	94	89	+ 5
10	2	78	117	101	99	92	-16
		100	146	123	100	109	-23

eter. In 5 cases, the 1 dose test was performed before the 2 dose test and *vice versa* in the other 5, since it has been shown by Lennox¹¹ that repeated blood sugar curves on non-diabetic individuals on successive days are progressively lowered.

The results of the 2 tests are set forth in Table 1. It will be seen that the fall in blood sugar in the second $\frac{1}{2}$ hour after the second dose of glucose in the 2 dose test is manifested only 5 times, whereas in the 1 dose test it occurs 7 times. Table 2 is a summary of the data from the last

following the second dose of glucose any more frequently than in the 1 dose test. This result is in direct contradiction to that of Exton and Rose who based their concept upon their finding that the blood sugar almost invariably fell after the second dose of glucose. A review of the literature reveals 2 additional series in which actual blood sugar values for both the 1 dose and 2 dose tests in the same individuals are available for comparison.^{2,16} Review of these figures reveals results similar to our own series, and data

calculated by us from these figures are set forth in Table 3. These data lead to the same conclusions as our own which is shown in Table 2 and add further confirmation to our thesis that the Exton-Rose test is based upon a misconception.

Magath and Berkson,¹² and a rise of 30 mg. per 100 cc. is allowed in the second $\frac{1}{2}$ hour by Gould, Altshuler and Mellen.⁶

In view of the data presented it is obvious that the 2 dose, 1 hour test follows the same type of curve as the con-

TABLE 2.—SUMMARY OF THE DIFFERENCES IN THE BLOOD GLUCOSE VALUES BETWEEN THE 30 AND 60 MINUTE SPECIMENS IN THE SAME INDIVIDUALS (AUTHORS' PRESENT SERIES)

Behavior of blood glucose	1 dose test			Exton-Rose test		
	No. cases	Average rise (mg./100 cc.)	Average fall (mg./100 cc.)	No. cases	Average rise (mg./100 cc.)	Average fall (mg./100 cc.)
Rise	3	6	..	5	12	
Fall	7	..	20	5	..	13

TABLE 3.—SUMMARY OF THE DIFFERENCES IN THE BLOOD GLUCOSE VALUES BETWEEN THE 30 AND 60 MINUTE SPECIMENS IN THE SAME INDIVIDUALS

Behavior of blood glucose	1 dose test			Exton-Rose test		
	No. cases	Average rise (mg./100 cc.)	Average fall (mg./100 cc.)	No. cases	Average rise (mg./100 cc.)	Average fall (mg./100 cc.)
Rise	4	+18	..	5	+15	
Fall	5	..	-23	4	..	-21
Rise	3	+23	..	7	+33	
Fall	7	..	-19	3	..	-39

First and second lines calculated from Brown (Med. Bull. Vet. Admin., 16 152, 1939; calculated from Groups II and IV: normal individuals).

Third and fourth lines calculated from Spellberg and Leff (J. Am. Med. Assn., 129, 246, 1945; calculated from Table 2).

Discussion. It is believed that sufficient evidence has been presented to prove that the Exton-Rose 2 dose, 1 hour glucose tolerance test does not differ physiologically from the conventional 1 dose oral glucose tolerance test using the same total dose of glucose. It is believed that it is misleading to consider the Exton-Rose 2 dose test as a separate entity. While Exton and Rose originally found that a majority of their blood sugar curves fell during the second $\frac{1}{2}$ hour after divided doses of glucose, this was not substantiated by others. The original criteria of Exton and Rose which required a fall or no rise of the blood sugar in the second $\frac{1}{2}$ hour of the test have been discarded in favor of criteria which do permit a considerable rise.^{6,12} This fact in itself would seem to vitiate the original concept of the Exton-Rose test. A single 1 hour blood sugar value of 160 mg. per 100 cc. has been adapted as a criterion by Matthews,

ventional 1 dose oral glucose tolerance test and the maximum permitted value at 1 hour in both tests is about the same. Thus, the original rise or fall phenomenon stressed by Exton and Rose and based on the paradoxical law of Allen ceases to be a determining factor. We believe that the crux of the matter is that the paradoxical law of Allen cannot operate (for the reasons previously set forth) in the short space of 1 hour when as large a dose as 50 gm. of glucose is given initially and again at $\frac{1}{2}$ hour.

In the diabetic the blood sugar will rise sharply during both the first and second $\frac{1}{2}$ hour of the test whether 1 dose of 100 gm. or 2 doses of 50 gm. are given. In the normal individual (whether 1 dose of 100 gm. or 2 doses of 50 gm. of glucose are given) the blood sugar curve may rise or it may fall in the second $\frac{1}{2}$ hour depending upon the speed with which the peak level is reached and upon the speed with

which the glucose regulating mechanisms become fully mobilized.

The reason for disagreements between the 1 hour, 2 dose and the conventional 1 dose test is that the criteria for the short 2 dose test are established at or near the peak of the blood sugar curve while the criteria for the longer test are usually judged most heavily at the 2 or 3 hour level when the glucose disposal mechanism is reaching equilibrium again. If experience demonstrates that the 1 hour level of a glucose tolerance test is superior to the 2 or 3 hour levels in diagnosing diabetes or other abnormalities of glucose metabolism, then this factor of the time for collecting the critical blood specimen is the important decision and not the giving of 100 gm. of glucose at once or in 2 closely spaced doses at as little as $\frac{1}{2}$ hour interval. At the present the weight of evidence does not favor the use of the peak value as the principal criterion but emphasizes the importance of using the 1 dose glucose tolerance test and following the blood sugar curve for 2 to 3 hours.¹⁵

Conclusions. 1. After giving 100 gm. of glucose in 1 initial dose the blood glucose curve is essentially the same as that obtained after giving 100 gm. in 2 divided doses of 50 gm. initially and 50 gm. at $\frac{1}{2}$ hour (Exton-Rose procedure).

2. The absorption of glucose from the gastro-intestinal tract and the response of the glucose regulating mechanism is not sufficiently rapid to permit a paradoxical response in the short space of $\frac{1}{2}$ hour. This is contrary to the hypothesis of Exton and Rose.

3. In view of the above 2 paragraphs, the physiologic hypothesis upon which the 2 dose, 1 hour (Exton-Rose) glucose tolerance test is based is unsound. The Exton-Rose test is not a separate physiologic entity and is not basically different from the 1 dose glucose tolerance test.

4. The interruption of the oral glucose tolerance test at 1 hour and the consequent use of the peak value as the principal criterion is much less accurate than the use of the 2 or 3 hour value of the glucose tolerance curve.

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ANALYSIS OF INCIDENCE AND CAUSES OF DEATH IN AN ACUTE PSYCHOPATHIC HOSPITAL

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THIS is a study of the causes of death in patients admitted to the Psychopathic Department of the Philadelphia General Hospital, from August 1943 to August 1944. In order better to understand this analysis, it is desirable to describe the type of patient admitted and the method of admission. The Psychopathic Department receives psychotic and unmanageable patients from all departments of its own hospital and any other hospital in the city which is unable to handle them, in addition to its own direct admissions. This applies particularly to senile patients who may get periods of agitation, forgetfulness, confusion, etc., when it is felt that they may do harm to themselves or others. Since there are no facilities for such close observation on medical or surgical wards, they are transferred to the closed wards of the Psychopathic Department and their treatment continued there. Therefore, the senile patients are classed together and treated as a separate group in this study, in order to make the remainder of the cases assume proper proportion.

These patients are studied, treated and finally disposed of, in several ways. They are treated if there is any specific therapy and if it is felt that they can be improved in a relatively short time. This includes electroshock therapy and malaria fever therapy. They may be transferred to private institutions, nursing homes or other appropriate facilities. Finally they may be committed to a state institution if considered chronically ill. The turnover of patients is relatively and necessarily rapid, so that, as indicated, this survey

deals with mortalities on an acute psychopathic department.

Statistical Data. In the period of 12 months being considered, there was a total of 3374 admissions. Of these, 827 fell in the senile group. The total number of deaths was 332, with 231 of these admitted primarily because of senile changes and death resulting from the usual geriatric causes, predominantly pneumonia. The mortality data are summarized in Table 1. Of particular interest to us are the patients included in the "Remainder" group.

Those in the "Remainder" group who died included 70 men, 31 women; 77 white, 23 colored, 1 yellow. Of the 101 cases, 59 were autopsied, including 20 which were coroner's cases (those dying within 24 hours after admission or in consequence of suspicious circumstances). The hospital stay averaged 13 days, with the range from less than 1 hour to 73 days. Age ranged from 23 years (a deteriorated epileptic) to 80 years (hypertensive cardiovascular disease), with an average of 53 years.

Table 2 lists the primary diagnoses and number of deaths in each group. The larger groups are discussed below.

Under the diagnosis of cardiovascular disease are listed all cases whose psychosis appeared to be due primarily to a circulatory disturbance and who subsequently died of it. It includes such cases as hypertensive encephalopathy with or without uremia, hypertension with cerebral accident, coronary occlusion, and cerebral embolus. (The senile group mentioned above included those cases of senile psy-

chosis, and psychosis with cerebral arteriosclerosis.) Of the total 33 cases, the primary psychotic manifestation of 13 (39.7%) was marked confusion or coma, 12 (36.3%) were agitated, and 8 (24%) were depressed, 7 of these having attempted suicide.

the use of intravenous vitamin therapy. Three cases (21%) had severe head injuries, 2 of these had subarachnoid bleeding and 1 of the latter showed laceration of brain at post. One case at autopsy showed generalized hypoplasia of all organs, including the circle of Willis;

TABLE 1.—DATA ON MORTALITY AMONG 1 YEAR'S ADMISSIONS TO PSYCHOPATHIC DEPARTMENT

	No cases admitted	Deaths	Mortality (%)
Total	3374	332	10.0
Seniles	827	231	28.3
Remainder	2547	101	4.0

TABLE 2.—CAUSES OF DEATH AMONG PSYCHOPATHIC ADMISSIONS OTHER THAN SENILE PATIENTS

	No. of deaths and % (101 cases)
Cardiovascular disease	33
Alcoholism	14
General paresis	13
"Agitation"	10
Tuberculosis	5
Neoplasm elsewhere with cerebral metastases	5
Brain tumor	3
Epilepsy	3
Bromism	3
Tuberculous meningitis	2
Suicide	2
Morphinism	1
Gangrenous cystitis	1
Digitalis toxicity	1
Marie-Stumpell disease	1
Malnutrition	1
Pachymeningitis hemorrhagica	1
Ruptured military aneurysm	1
Alzheimer's disease	1
Total	101

TABLE 3.—DATA ON THE CHIEF CAUSES OF DEATH AMONG NON-SENILE PSYCHOPATHIC ADMISSIONS

	No deaths	No admissions	Mortality (%)
Cardiovascular disease	33	273	12.1
Alcoholism	14	479	2.9
General paresis	13	235	5.5
Epilepsy	3	83	3.6
Bromism	3	10	30.0

Table 3 shows the chief causes of death among the non-senile patients.

The alcoholics are grouped together and include all cases whose admission to the hospital was primarily because of some form of alcoholism. These include 7 chronic alcoholics, 5 cases of delirium tremens, and 2 of alcoholic hallucinosis. The largest number, 8 patients (57%), succumbed to pneumonia. This group is undoubtedly smaller than formerly because of the advent of the sulfa drugs and

1 had had a cerebrovascular accident, and 1 who had been drinking a combination of alcohol and ether showed acute and chronic hepatosis with hepatic insufficiency. There was 1 patient with Korsakoff's syndrome due to alcohol, who went downhill rapidly despite therapy and died, but no autopsy was performed.

In the group with general paresis, all 13 had positive spinal fluid findings for syphilis. Seven were old known cases of general paresis, deteriorated on admis-

sion; 3 were quite agitated when they first came to the hospital and became rapidly worse; 1 developed a sudden elevation of temperature (not receiving fever therapy) and died. The postmortem examination showed syphilitic aortitis and pulmonary emboli. One patient was treated with deep Roentgen ray therapy according to the method of F. Bering, and 11 days after the last treatment developed convulsive seizures and died. No patient of this group was on malaria fever therapy at the time of death.

The 10 cases listed as "agitation" are grouped together because all were admitted to the hospital in agitated states, all were studied as thoroughly as possible but no definite medical diagnosis could be established, and all died without obvious cause; 5 were examined postmortem and none showed anything except terminal bronchopneumonia and chronic passive congestion of other organs. One was a coroner's case because he died in less than 24 hours after admission into the hospital. There were 4 women and 6 men, ranging in age from 25 to 60 years, with an average of 44. The stay in the hospital varied from a few hours to 56 days. There were 2 Negro and 8 white patients. On admission to the hospital the provisional diagnosis was schizophrenia for 5, agitated depression for 3, reactive depression for 1, and toxic psychosis for 1.

Two of the "agitated" patients died rather acutely. The first was a 25 year old Negro male who became irrational at work, heard voices and was very agitated and noisy. On admission to the hospital he was restless, confused and very difficult to control. Physical examination was essentially normal. A mild sedative was administered but the agitation continued and he died approximately 6 hours after admission. A postmortem examination by the coroner revealed no specific cause of death. The second was a 49 year old white male who also became agitated while at work. This continued until he was hospitalized. On the ward he was extremely restless and irrational. Past

history was non-contributory. Physical examination showed a temperature of 105° F., pulse 125, respirations 36. The leukocyte count was 28,000, with 81% neutrophils and 19% lymphocytes. The agitation and restlessness continued and the patient died 2 days after admission. A postmortem was done and all organs were essentially normal, including the brain which showed only "acute and chronic passive congestion."

In the remaining 8 "agitated" cases, death occurred in from 12 to 56 days. The youngest of these was a 27 year old white male who had been acting strangely for 2 to 3 weeks prior to hospitalization. He had been going about ringing doorbells, had been unable to sleep and was continually masturbating. He was markedly hyperactive and agitated on admission. Physical examination was essentially normal. Temperature on admission was 99° F., then fluctuated irregularly as high as 106°. The pulse was 110 on admission and rose at times to 130. Respirations were 25 on admission and fluctuated to 45. The leukocytes numbered 14,900, with neutrophils 94% and lymphocytes 6%. Blood urea nitrogen was 27 mg. per 100 cc. The patient's course in the hospital was progressively downhill and he died on the 12th day. Postmortem examination showed only "chronic passive congestion," including the brain.

A second "agitated" patient who lived for 12 days was a 38 year old Negress who was admitted to the Tuberculosis Department because she appeared toxic and had a fever ranging from 102° to 104° F. She became hallucinated, appearing like a case of delirium tremens, expressed many bizarre complaints, and had to be transferred to the closed wards. Physical examination was essentially negative and the results of all laboratory studies, including chest Roentgen rays, were normal. This activity and agitation continued and the patient died on the 12th day. An autopsy was not obtained.

A 43 year old white married male was admitted to the hospital because he had

begun to worry about an affair he was having with a girl he had met in church. He began to hallucinate, was taken to his doctor and became upset and struck him. Upon admission to the hospital he was actively hallucinating and expressed many delusional ideas. He was quite agitated and restless. Physical examination was essentially negative. Sedatives were given and included intravenous sodium amytal (the latter for both sedation and abreaction without obvious effect). The increased activity persisted. The temperature rose to 103° F., pulse and respirations increased. All laboratory studies including agglutination tests for typhoid and the paratyphoid groups were negative and the patient died on the 13th day. No autopsy was obtained.

The 3 cases of "agitated depression" ran a similar course to those described above. Two of these were given electroshock therapy. One of these 2 had been given a complete course and was sent home, but returned quite agitated and disoriented, remained that way for 10 days and died. Postmortem examination showed only a "terminal bronchopneumonia." The other was given 11 electroshocks, but agitation and depression persisted. Three days after the last treatment, and while still agitated, the patient developed Cheyne-Stokes respirations, became cyanotic and died despite treatment. Unfortunately no autopsy was obtained.

The remaining 2 "agitated" patients were females, 1 of whom appeared schizophrenic on admission, the other suggesting a reactive depression. The former had a history of previous mental breakdown 25 years before the present admission. This time she was excited, agitated and had shown violent behavior since the birth of a child 4 months ago. She had a persistent fever but all studies, including agglutination tests, were negative. Sulfa drugs were tried empirically but the agitation and restlessness persisted and patient died. The postmortem examination showed only bronchopneumonia.

The last case of this group was a 50 year

old white female who became 'jittery' and worried about her alcoholic husband. She became negativistic, would not eat and appeared malnourished on admission. She was quite agitated, required restraint and tube feeding. She was extremely uncooperative, became mute and died on the 21st day.

In the group with tuberculosis, 2 attempted suicide unsuccessfully and were admitted to the wards as cases of depression. One patient was admitted because of agitation; 1 was comatose on admission and 1 case, brought to the hospital as mild senile psychosis, showed tuberculous pneumonia at necropsy.

In the 5 patients with metastatic brain tumor the primary growth was bronchogenic carcinoma in 2, and carcinoma of stomach, larynx and cervix respectively in the other 3. Four of the 5 were admitted because of agitation, 1 was confused and showed marked organic deterioration. All 5 cases were examined at autopsy.

Unfortunately, of the 3 patients believed to have primary brain tumor, only 1 came to necropsy. The tumor proved to be an astroblastoma of splenium corpus callosum. The other 2 patients had definite clinical findings pointing to intracranial lesions, cerebellar in 1, and in the right temporal lobe in the other.

Of the 3 epileptics, 1 was a 23 year old Italian woman, markedly deteriorated, agitated and confused, who died 2 days after admission. The second was a 61 year old woman who had been a known epileptic for 25 years, was admitted to another hospital as suspected intestinal obstruction, became overactive, irrational and disoriented, and died 7 days after admission. The last case, a known epileptic, was a 28 year old white man markedly agitated and confused on admission, with a rectal temperature of 107° F. (found to have a subarachnoid hemorrhage of unknown etiology), and died on the 4th day (no autopsy).

The 3 cases of bromism had blood bromides (on admission) of 420, 340 and 265 mg. per 100 cc. respectively. Two were

known to be heavy alcoholics. All 3 were comatose on admission, 2 were started on sodium chloride, the other died before treatment could be instituted, all dying within 1 week of admission to the hospital.

The 2 patients with tuberculous meningitis were both stuporous on admission and died 5 days later. The 2 cases of successful suicides were both involuntal melancholia. The remainder of the cases are as listed.

Of the so-called purely functional psychoses, there were no deaths among the clear-cut schizophrenics (although some of the "agitation" group may fall in that class), none was a proven or known manic-depressive psychosis, and there were 2 successful suicides among the involuntal melancholics.

Summary. 1. A survey of the causes and incidence of death in an acute psychopathic department was made. Of 3374 patients admitted in 1 year, 332 died. Senile patients numbered 827, of whom 231 died. Of the 2547 non-senile patients, 101 died. The causes of death in the latter group are analyzed.

2. The commonest cause of death was cardiovascular disease.

3. Alcoholism and general paresis were about equal as the next commonest causes of death.

4. Ten per cent died of "agitation" of unknown etiology.

5. Tuberculosis and neoplasm (both primary in the brain and metastatic) were next in frequency.

We wish to acknowledge our indebtedness to the Pathology Dept., especially Dr, Helena Riggs and Dr. William Ehrich.

THE EFFECT OF AMINOPHYLLINE ON THE PROTHROMBIN TIME IN MAN

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In 1944, Link and his co-workers published a series of observations on the action of certain xanthines on the prothrombin time of several experimental animals.^{2,3,4} They were able to demonstrate that large single doses of caffeine, theophylline, theobromine, aminophylline, and other xanthines, when given orally to the dog, rabbit or rat induced a state of hyperprothrombinemia which was readily detectable in diluted (12.5%) plasma. When these animals were given 3,3'-methylenebis - (4 - hydroxy - coumarin) with the methylxanthines, the expected hypoprothrombinemia was partially neutralized. Relatively large therapeutic doses of aminophylline (12, 24, 36 mg./kg. by mouth daily) produced a hyperprothrombinemic effect in dogs detectable in 2 to 5 days, and maintained for periods as long as 30 days. The authors suggested that, "Since our results . . . indicate that the methylxanthines not only render the blood hypercoagulable, but also counteract such a potent hypoprothrombinemic agent as 3,3'-methylenebis-(4-hydroxy-coumarin), it is conceivable that their use in man might augment the tendency for thrombus formation."² It was to determine the effect of therapeutic doses of aminophylline on the prothrombin time of man that this study was undertaken.

Method. Many workers have advocated the use of diluted (12.5%) plasma in order to detect small changes in prothrombin times.^{2,3,4,6,7,8,9} The determinations of prothrombin time were done by the Quick method,⁵ as standardized by Aggeler, *et al.*¹

All experiments were performed on fasting blood, and were completed within 2 to 6 hours after the specimens were drawn. The subjects were young, adult, male patients with mild bronchial asthma, in a Naval Hospital. They were under no other drug therapy at the time of the study. Five cc. of blood were mixed with the dry oxalate mixture of Heller and Paul (4 mg. potassium oxalate and 6 mg. ammonium oxalate). After centrifugalization the plasma was diluted to 12.5% with 0.85% sodium chloride. In all experiments a single specimen of dehydrated human brain, prepared by acetone extraction and stored in an evacuated desiccator, was used.* Under these conditions, 0.005 M. calcium chloride solution was found to produce optimal results. All determinations were made in triplicate at 37° C. and the results averaged.

Results. 1. *Normals.* (a) Prothrombin times were determined on 10 normal individuals, using 12.5% plasma. In this series, the mean was 24.5 seconds, the range 22.7 to 26.8 seconds, and the standard deviation plus or minus 1.4 seconds. These values are comparable to those found by other workers using this method.⁵

(b) Prothrombin times were determined on 10 separate specimens of blood from a single individual. In this series the mean was 24 seconds, the range 19.8 to 25.8 seconds, and the standard deviation plus or minus 1.7 seconds. Using 2.5 as the level of significance, it can be shown that a difference of plus or minus 4.3 seconds (from the mean) is required for the variation to be significant. This means that a change in prothrombin time in any given series on 1 individual would have to be

* This material was supplied by Dr. Paul M. Aggeler, to whom we wish to express our thanks.

greater than 4.3 seconds in order to be certain that the variation was not due to chance.

2. *Effect of Small Oral Doses of Aminophylline.* A group of 5 patients were given aminophylline, 0.1 gm. orally 3 times a day for 7 days. Prothrombin determinations before, during, and after therapy showed no significant change in the prothrombin time (Table 1). None of the patients developed toxic symptoms.

prothrombin times were determined before, during and after therapy. Again, no significant change was observed (Table 3).

Comment. It has been shown that, in man, hyperprothrombinemia can be detected using 12.5% plasma.^{6,7,8,9} In this small series, no hyperprothrombinemia could be demonstrated following administration of aminophylline. It would seem, then, that the fear that aminophylline may produce hyperprothrombinemia (and

TABLE 1.—PROTHROMBIN TIME IN SECONDS OF 12.5% PLASMA FOLLOWING AMINOPHYLLINE IN ORAL DOSAGE OF 0.1 GM. 3 TIMES DAILY FOR 7 DAYS

	Patient: W. K. A.	K. W. B.	J. S. B.	J. A. C.	T. W. C.
Before aminophylline	26.8	23.3	22.2	23.2	22.0
Before aminophylline	26.2	24.0	22.5	24.8	23.5
On 5th day of therapy	23.3	24.5	24.7	22.8	27.3
1 day after discontinuance of therapy . .	24.3	26.0	23.8	25.2	27.7

TABLE 2.—PROTHROMBIN TIME IN SECONDS OF 12.5% PLASMA FOLLOWING AMINOPHYLLINE IN ORAL DOSAGE OF 0.5 GM. 3 TIMES DAILY FOR 2 DAYS

	Patient: M. K. A.	H. J.	E. H. P.	H. L. G.*	J. M. C.†
Before aminophylline	24.3	25.5	25.3	..	27.7
Before aminophylline	24.0	25.2	24.0	24.5	28.7
After 1 day of therapy	23.3	26.8	26.8	25.5	27.0
After 2 days of therapy	22.5	25.5	24.7	21.3	
3 days after discontinuance of therapy . .	23.3	24.5	24.0	24.7	28.3

* This patient received aminophylline, 0.5 gm. intravenously, on 1st day of therapy in addition to oral dose.

† This patient received 0.5 gm. aminophylline 3 times a day for 1 day only.

TABLE 3.—PROTHROMBIN TIME IN SECONDS OF 12.5% PLASMA FOLLOWING INTRAVENOUS ADMINISTRATION OF 0.5 GM. OF AMINOPHYLLINE

	Patient: M. K. A.	H. J.	E. H. P.
Before aminophylline	22.0	23.8	22.8
Before aminophylline	24.0	22.7	24.3
3 hours after administration	24.3	24.7	22.4
24 hours after administration	24.3	25.5	25.3
72 hours after administration	24.0	25.2	24.0

3. *Effect of Large Oral Doses of Aminophylline.* A group of 4 patients were given aminophylline, 0.5 gm. orally 3 times a day for 2 days. Prothrombin determinations before, during and after therapy showed no significant change in the prothrombin time (Table 2). All of the patients experienced minor toxic symptoms of anorexia, slight nausea, and nervousness.

4. *Effect of a Single Intravenous Dose of Aminophylline.* Three patients were given 0.5 gm. aminophylline intravenously and

an increased tendency toward thrombus formation) is unwarranted, and does not constitute a contraindication to the therapeutic use of the drug.

Summary and Conclusion. Aminophylline administered orally and intravenously in full dosage to a small series of human subjects produced no significant change in the prothrombin time.

The therapeutic use of the drug therefore probably does not involve any increased threat of intravascular clotting.

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PROGRESS

OF

MEDICAL SCIENCE

DERMATOLOGY AND SYPHILOLOGY

UNDER THE CHARGE OF
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TUMORS OF THE SKIN

PART II. A REVIEW OF RECENT LITERATURE*

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Epithelioma (Cutaneous Carcinoma). For ordinary considerations cutaneous epitheliomas are divided into 2 groups, those of basal cell and prickle or squamous cell or epidermoid carcinoma. This distinction might appear of little significance but, as indicated above, the differences in course, location, treatability, metastasis, etc., make this distinction of practical if not theoretical, importance.

Basal cell epitheliomas vary in size from small to large, are chronic, with a slowly growing superficial to deep nodule or ulcer and a rolled pearly, telangiectatic border, the center of which is usually covered with a crust, removal of which causes bleeding. This type of lesion occurs predominantly on the anterior portion of the face and forehead (Glasunow;¹⁰⁰ McFarland, Ciccone and Gelehrter;¹⁸² Schrek;²⁶² Sutton^{287c}), but it may occur anywhere on the body. Histologically, there is a varying arrangement of strands, like a lattice network, a glandular arrangement, or a solid growth of basal cells arising from the basal cells of the epidermis, rarely from the basal cells of the dermal appendages. Montgomery^{193a} has made an

extensive study of the histogenesis of these tumors and his classification of epithelioma presumably of basal cell origin is worth serious consideration.^{193c}

Basal cell epitheliomas may be benign, but in time local destruction may occur involving both cartilage and bone. They do not metastasize and those which seem to have metastasized will turn out on examination to be basal squamous or squamous cell epitheliomas (Montgomery^{193a,c}). Pigmented basal cell cancers may occur. The pigment (melanin) is deposited in and between the cells of the tumor. Examples of the pigmented basal cell epithelioma have been reported by Eller and Anderson,⁸³ Becker,^{23a} Montgomery,^{193c} and by Quiroga and Helman.²⁴³ These latter authors believe that pigmented lesions are not rare. On superficial examination, these pigmented basal cell epitheliomas may resemble pigmented nevi or melanomas; but they are much firmer and more indurated than pigmented nevi and of perhaps a more brownish rather than bluish color more characteristic of melano-epithelioma. They also do not show extension along the lym-

* The first part of this review appeared in the April issue.

phatics. On the other hand, Nisbet²¹⁰ has reported another case of the type originally described by Nomland^{212b} of multiple basal cell epitheliomas originating from congenital pigmented basal cell nevi. These cases had the gross clinical appearances of pigmented nevi. Angiomatous forms of basal cell epithelioma have been reported by Lamb, Geschickter and Lain.¹⁴⁵ Basal cell epitheliomas may also resemble other dermatoses such as, for example, senile sebaceous adenomas, a benign lesion also named by Nomland.^{212a} (See above.)

Montgomery^{193f} states that about 20% of neoplasms diagnosed clinically as basal cell epitheliomas are in reality transitions between basal and prickle cell lesions, the so-called basal squamous cell epithelioma. While there is no clinical difference between basal cell and the transitional form, histopathologically they are characterized by a definite picture of partial pearl formation, with brightly staining colloidal-like centers, light-staining intermediary and prickle cells and darker staining basal cells at the periphery. They tend to infiltrate the tissue in narrow strands, both going down and reaching out at the margins of the lesions. This fact indicates the need for wider excision than for basal cell epitheliomas.

Treatment of basal cell epitheliomas is by excision, electrodestruction or radiotherapy. The type of therapy depends on the size and location of the lesion. Warren and Lulenski³²⁰ found basal cell epitheliomas to have a less formidable prognosis than have epidermoid carcinomas from the standpoint of mortality, but they have a very little better outlook as far as 5 year cures are concerned. This is a point that should be stressed, since the clinician usually regards basal cell cancer as a relatively benign lesion and one of small importance. A visit to the cancer wards of any large city general hospital will soon dispel any doubt about the disabling propensities of this type of tumor.

Prickle cell carcinoma usually presents

a wider and more indurated border than basal-cell epitheliomas. Senile keratoses are frequently forerunners of this type of lesion. They are most apt to occur on the exposed parts of the body. They may attain large size and be destructive. Carcinoma of the mucous membrane is always of this variety. They may resemble other disorders, especially gumma, if situated on the lip or tongue. Broders, as previously stated, classifies these tumors in 4 grades depending on the amount of differentiation of the individual cell, mitosis and pearl formation. Squamous cell carcinoma has a decided tendency to metastasize, especially Grades 3 and 4 of Broders. Treatment should be radical and the type will depend on the situation and type of lesion. End-results are worse than in basal cell epithelioma, which has the advantage of slow development, non-metastasis and occurring on an accessible situation. Squamous cell cancer is thoroughly discussed in the surgical and radiologic literature.

Calcifying Epithelioma (Malherbe's Epithelioma). This type of tumor had received little attention in the American literature until Ch'in's⁵⁴ article in 1933 and Sutton and Sutton's^{286a} report of a case in 1935. The distinguishing features of this tumor as outlined by the Suttons are as follows:

"(a) The spherical shape. (b) The size generally of from 1 to 3 cm. (c) The location under the derma, fixed to the skin above and freely movable over the deeper tissues. (d) The hardness, which may be almost bony. (e) The occurrence (in order of frequency) on the head, arms, forearms and back, then elsewhere. (f) The occurrence in persons of any age, but often in youth or in early adult life. (g) The spicular, gritty surface of the gross section. (h) The component bands of epithelium, which hornify, calcify and become engulfed by giant cells. (i) The encapsulation and continuity with the capsule of a dense fibrous stroma remarkable for the giant cells in its structure. (j) Its behavior as a benign

neoplasm or local growth. (k) Its amenability to local excision with permanent cure."

Highman and Ogden¹¹⁹ (1944) believe the most characteristic feature to be eosinophilic degeneration of the epithelium which they think is a form of dyskeratosis.

Malherbe and Chenantis,¹⁷⁵ in 1880, were the first to describe this type of tumor as calcifying epithelioma of sebaceous glands. Since then reports have appeared, especially in the French and German literature. Ch'in, in 1933, reported on 10 tumors with this diagnosis gathered from over 22,000 surgical specimens covering a 15 year period. From a study of the literature he collected 116 additional cases. Coté⁶³ reported 12 cases in 1936. Highman and Ogden¹¹⁹ reported on 1 probable and 11 definite tumors of this type gathered from a series of 24,185 surgical specimens received over a period of 15 years.

This tumor may occur at any age. In Highman and Ogden's series, in which most of the diagnoses were on a clinical basis, the age range was between 17 months and 66 years. In a recent series of 13 cases reported by Muehlon, which clinically were thought to be mostly dermoids or atheromatous change, the lesions first appeared in 8 persons less than 20 years of age, in 4 persons 20 to 30 years old and in 1 at the age of 65 years. Highman and Ogden found the cases to occur in 4 females and 8 males, a ratio of 1 to 2. Muehlon, however, found 9 in females and 4 in males.

The origin of the tumors is not clear. According to Highman and Ogden they have been variously regarded as endothelioma, atheroma, basal cell carcinoma, a derivative of an epithelial rest, a result of traumatic epithelial inclusions, cholesteroloma and dermoid and epidermoid cyst. Malherbe suggested that the lesion results from abnormal proliferation of the lining wall of a sebaceous cyst. Kaufmann¹⁵⁹ stated that the origin may be in preformed glands as well as in deep-

lying (dystopic) epithelial cells, probably misplaced anlage material. Coté expressed the belief that it was a lesion sui generis possibly derived from the anlage of a sebaceous gland in which the final growth occupies an intermediate position between epidermoid cyst and basal cell epithelioma. Muehlon believes these lesions are hamartomas, the peculiar feature of which is that the aberrant basal cells are potentially destined to eventual spontaneous necrosis. Highman and Ogden suggest that these tumors may have an origin from or similar to that of hair follicles.

Multiple Superficial Epitheliomatosis. Much of the literature on this subject has been recently reviewed by Weisman and Medalia.³³⁰ Multiple superficial epitheliomatosis, as distinct from morphea-like and superficial types of arsenical epitheliomas, was first clearly described by Graham Little¹⁶² in 1923 under the name of "erythematoid benign epithelioma." Wise³³⁸ claimed that prior to this time it was called multiple benign superficial epithelioma. In recent years there has been considerable confusion as to what constitutes this process clinically and histologically. Savatard^{259a} concluded from a survey of the literature of a 60 year period that, because of the protean clinical and histologic manifestations, variants of this dermatosis have been described as separate entities. He maintained that mammary and extramammary Paget's disease of the skin, erythroplasia of Queyrat, and Bowen's precancerous dermatosis were different forms of the same disease. He found in his experience that they all resembled a psoriatic plaque and he accordingly proposed the name, "psoriasiform carcinoma of the skin" for them. This is not in keeping with the experience of Montgomery^{193b} and others, which leads to a distinction between superficial epitheliomatosis and the aforementioned states. Bowen's disease is, according to Montgomery, in reality a squamous cell epithelioma *in situ*. Dörffel who found 18 cases among 200 epithelial

skin tumors studied histologically between 1925 and 1935 (University of Königsberg) felt that there were few characteristic features in these lesions. He differentiated them from lesions of eczema, psoriasis, lichen planus, syphilis, lupus erythematosus, Bowen's disease and Paget's extramammary dyskeratosis. Biopsy is necessary to establish the diagnosis. The cause of multiple superficial epitheliomatosis is unknown. Ingestion of arsenic was frequently suggested to explain the lesions (Tauber and Goldman;²⁹³ Hopkins and Van Studdiford;¹²⁵ Ayres;¹⁶ Anderson;¹¹⁶ Wright and Friedman³⁴¹). It is more frequent in women than in men (14 to 4, Dörffel; Madsen¹⁷⁴). Madsen believes the lesions are unicentric in origin.

The lesions may be dry or eczematoïd in type. They first appear on the trunk as erythematous, smooth, flat, irregular, shiny macules and papules, which gradually enlarge and coalesce to form plaques 2 or 3 cm. or more in diameter. A fully developed case has multiple reddish to violet discoid patches of variable size up to 20 cm. in diameter. Many of the patches are smooth and not infiltrated. A certain amount of scaling, oozing and crusting may be a feature. There is a characteristic fine thread-like, somewhat elevated border surrounding the lesions. There may be central healing consisting of a white atrophic surface with some telangiectasia in some of the long-standing lesions. They may persist with little change for as long as 40 years.

Madsen¹⁷⁴ in his monograph based on his own 10 cases and the literature adds new features, such as melanin in the plaques and the occurrence in younger persons. He believes the pigment is the result of inflammatory reaction in response to tumor. The lesions are single in 42% of the cases; they may in rare instances exceed 200 to 400 (Pautrier and Achambault; van der Meiren). They may occur anywhere except on the palms, soles, fingers, toes and genitalia. The dorsum of the hand is a rarely affected site. The course is benign, metastases are rare,

though local recurrences are frequent. We have seen recurrences at the margins of treated lesions even in some which have sustained an irradiation reaction (atrophy and telangiectasia).

Metastatic Cutaneous Malignancy. Cutaneous carcinoma may result from carcinoma situated anywhere in the body, and may occur at any age—even in the newborn²⁵—but usually late in life. Such involvement may arise: (1) by extension by direct invasion from adjacent tumors; (2) by extension through the lymphatics (Sampson-Handley's "permeation"); (3) dissemination through lymphatics with or without involvement of the lymph nodes between the primary growth and the metastatic lesion; (4) the dissemination through the blood stream, as occurs in many cases of cutaneous metastasis. Only the last two are metastases in the strict sense of the word. The incidence of cutaneous metastasis of malignant disease is not high. In a study devoted to this subject Olive Gates⁹⁶ found metastases of the skin in 93 (0.1%) of 82,298 specimens; 29 (0.04%) from 80,000 surgical specimens, and 64 (2.7%) from autopsy specimens of 2298 malignant tumors. Gates regards the autopsy series as probably the more representative figure. Her figures are higher than those in the literature on the frequency of secondary skin tumors (0.01 to 0.7% for all types of cases malignant and non-malignant). Of 2031 carcinomas in the autopsy series, 43 (2%) had skin metastasis. In the group from the literature the number of skin metastases from mesenchymal and epithelial tumors is practically the same, 176 sarcomas against 173 cases of carcinoma. Carcinoma of the breast is the most important primary tumor site in this respect, producing as many secondary skin tumors as all other types together. Among 58 metastatic tumors, 25 originated in the breast; however, collected cases from the literature showed only 8 out of 173 metastatic carcinomas primary in the breast. This may be due partly to the fact that skin

tumors from breast carcinoma are so common as to excite little interest and partly to the common assumption that they result from lymphatic permeation and are not true metastases. The remaining 24 cases in Gates' series arose from 14 different organs: 4 from the lung; 3 each from the uterus and kidney; 2 each from the stomach, rectum and pancreas; 1 each from the liver, adrenal, thyroid, tonsil, jaw, urethra, prostate and penis. From the literature, carcinoma of the stomach (54 cases) produced the majority of secondary skin tumors. After the stomach the order of frequency is: uterus, 17 cases; lung, 15; large intestines, 13; kidney and ovary, 10 each; esophagus, 5; liver, 4; urinary bladder, 3; tonsil, pharynx, trachea, adrenal, thyroid, 2 each; nose, larynx, parotid, tongue, penis and gall bladder, 1 each. In both series excepting breast and stomach, carcinoma of lung, uterus and kidney are the most important sources of skin metastases, the incidence varying from 5 to 9%. If carcinoma of the breast is withdrawn from both series, the incidence for different sources of epithelial skin metastases is practically unchanged for the literature group, but is considerably increased for carcinomas of Gates' series. The stomach has long been considered the most important source of skin metastasis, but the statistics from the literature are contradictory on this point, and do not altogether substantiate this assumption. From Gates' analysis it appears obvious that carcinoma metastatic in the skin is frequently not recognized. The importance of these metastatic lesions is that they are often the first evidence of the existence of malignancy and also of metastasis. These cutaneous metastases are not necessarily immediate precursors of death. They are of limited prognostic value, since the behavior of the skin tumors does not necessarily indicate rate of growth of the primary tumor. The gross appearance of skin metastases is not distinctive. They have a predilection for certain sites, especially the chest, in

the axilla, on the abdomen and in the perigenital region. In some cases the secondary growth may overlay the primary tumor. The scalp is rarely affected by metastasis; when found it must be distinguished from turban tumors and carcinomas of various dermal appendages (Montgomery and Kierland;¹⁹⁹ Ronchese^{252b}). Ovarian carcinoma with metastases to the skin has rarely been reported. Urbach, Waldow and Stamm,³¹¹ in an interesting case report of diffuse metastatic lesions from an ovarian carcinoma, review the literature and note only 8 cases besides their own, the first in the American literature. This case is especially interesting because of erysipelas-like features resembling the condition known as "erysipelas carcinomatosum" (Lee and Tannenbaum;¹⁵² Rasch;²⁴⁶ Pfahler and Case²²⁹). Unfortunately the authors did not mention Gates' paper in which 10 cases of ovarian carcinoma with cutaneous metastases were gathered from the literature, although she had no personal cases. Since Gates does not detail the source of her cases from the literature, Urbach and his associates' case still appears to be the first in the American literature. Nicholas²⁰⁹ reported a case of acanthosis nigricans in which deep cutaneous and subcutaneous metastases from a retroperitoneal lymphosarcoma were observed histologically. This case suggested the advisability of examination of the deepest parts of the corium and subcutaneous fat in future histologic studies of acanthosis nigricans. Nödl has recently reported skin metastases of a malignant hypernephroid tumor.^{211b}

In Gates' group of 442 cases of cutaneous metastases, there were 200 mesenchymal tumors. Lymphomas and leukemias make up 94% of the mesenchymal tumors and 43% of the entire group. These mesenchymal tumors made up 23% of her autopsy group. Of these the lymphomas and leukemias are the most important groups, secondary skin tumors being present in 8 (9%) of 85 lymphoma autopsies and in 31 (6%) of 50 leukemia

autopsies. A little over one-half of the lymphomas in the series of leukemia cutis were from autopsies. This is explained on the basis that in lymphoma cutis the primary disease is less obvious than in leukemia and diagnosis frequently depends on biopsy. The lymphoma-leukemia group was responsible for most of the widespread cutaneous metastases (53 of 69 instances were in this group).

Cutaneous Carcinoma Arising From Various Cutaneous Disorders. A number of cutaneous diseases have been secondarily affected by carcinoma. Among them are xeroderma pigmentosum, rhinophyma (Novy²¹⁴); Darier's disease (Carache⁴⁷); sebaceous cyst (Stone and Abbe²⁷⁹); psoriasis (arsenic?) (Wright and Friedman³⁴²); lupus vulgaris (0.5 to 1% of cases) (Ormsby and Montgomery²¹⁶); acrodermatitis chronica atrophicans (Pack and Wuester²²³); radiodermatitis; osteomyelitic cavity or sinus (Steward, Obermayer and Woolhandler;²⁷⁶ Bereston and Ney²⁸); lupus erythematosus, granuloma inguinale and lymphogranuloma venereum. One must be careful to be certain in some of these supposed carcinomatous complications that he is not dealing with pseudo-epitheliomatous change. Only lupus erythematosus and lymphogranuloma venereum will be detailed here. The mechanism of the carcinomatous change is not clearly understood in most of the disorders listed. Trauma, arsenic, irradiation (sun, Roentgen ray) are among the proposed explanations.

(a) *Epithelioma as a Complication of Lupus Erythematosus.* In reviewing this complication of lupus erythematosus, Ambrosetti⁸ stated that epithelioma is not exceptional in lupus erythematosus, but is not as frequent as is the case of lupus vulgaris. Dicke⁷² collected 50 cases, 3 personal; Veiel³¹² collected 90 cases; Tischnenko²⁹⁹ collected 91 cases (1 basal cell), remainder were prickle cell carcinoma. These cases are supposed to occur most often after prolonged irritation (pyrogallie acid) or injudicious amounts of radiotherapy.

Uhlmann and Schambye³⁰⁸ believe that cancer was not rare in this dermatosis, but denied that Roentgen rays facilitated its development. Louchtchitzky¹⁶⁵ observed 400 patients of whom 15% (60) were irradiated with Roentgen rays, 6 (4 women and 2 men) presented epitheliomas (10%). These appeared 6 months to 7 years after the application of the roentgenotherapy. There were no cases of cancer among those who did not receive Roentgen irradiation. He believed that these patients had hypersensitivity to Roentgen rays and considered it a dangerous type of treatment which should be avoided in the treatment of lupus erythematosus. Pautrier²²⁵ cited 2 cases of prickle cell cancer of the lips. Le Coulant¹⁵¹ presented a case of precocious spirocellular epithelioma of grave prognosis in a patient who had had no previous radiotherapy. Kogoj¹⁴⁵ encountered only 3 cases of epithelioma among 385 patients with lupus erythematosus. In 315 patients of Puente²³⁸ there were only 2 examples of epithelioma; 1 following Roentgen therapy, the other occurring without previous Roentgen irritation.

(b) *Malignancy and Lymphogranuloma Venereum.* Deibert and Greenblatt⁷¹ believe that genital malignancy may be co-existent with, simulated by, or a direct sequel of one of the venereal diseases. They believe the association of the malignancy and venereal disease is too frequent to be coincidental. Etiologic relation has been suspected by a number of authors as Pund, Greenblatt and Huie;²³⁹ Cardwell and Pund;⁴⁸ Liccione;¹⁵⁹ David and Loring;⁶⁹ Lisa;¹⁶⁰ Reeves;²⁴⁸ Greenblatt;¹⁰⁹ Smith;²⁷⁰ Taussig.^{295c} Lately Guzman¹¹⁵ reported vulvar involvement of lymphogranuloma venereum and carcinoma. Similar associations were reported by Cardwell and Pund; Pund, Greenblatt and Huie and Deibert and Greenblatt. Binkley and Derrick³³ reviewed the literature on the association of squamous cancer with anal manifestations of lymphogranuloma venereum. Among 87 cases of squamous cancer of the anus and rectum at the

Memorial Hospital in New York, study revealed 8 positive and 11 negative Frei tests among 19 tested. The remainder of the cases were treated prior to the routine employment of the Frei test. It is necessary to point out that the Frei test, made with all types of antigens, cannot, in the Reviewer's opinion, be relied upon implicitly as an indication of the existence of lymphogranuloma venereum.

Malignant Melanoma. Without entering into the long-standing controversy as to the epidermal *versus* mesodermal region of these tumors, we shall discuss them under the non-committal name of malignant melanoma, as proposed by Becker,^{23b} but with the conviction based on the best American dermatologic authority that they are really probably epidermal origin (melano-epithelioma). Among the recent papers on this subject are the general discussion of Becker (1936); Butterworth and Klauder on malignant melanomas arising from moles;⁴⁵ Netherton²⁰⁶ on cutaneous and nervous system involvement; Reese^{247a} on precancerous melanosis and diffuse malignant melanoma of the conjunctiva and his later study;^{247b} Dixon;⁷⁴ Daland and Holmes;⁶⁷ Pack and Adair²²¹ on subungual melanoma; Moersch, Love and Kernohan;¹⁹¹ Taussig and Torrey;^{295d} Sweet and Connerty;²⁸⁸ Howes and Birnkrant;¹²⁸ Tod;³⁰⁰ Driver and MacVicar.^{77a}

Malignant melanoma is essentially a disease of the white race. It occurs more frequently in males. These lesions may occur at various ages, but the average is around 50 years. For example, Howes and Birnkrant (1943) found an average age of 52.4 years (youngest 22 years) among their 32 patients. Taussig and Torrey found an age range of 5 to 86 years, with an average of 48.7 years. Sweet and Connerty presented a case with antenatal metastasis. The process usually begins as a solitary blue black or steel blue flat or elevated nodule, plaque or tumor of several millimeters to several centimeters in diameter. Occasionally the color may be a lighter brown, especially when a pigmented nevus undergoes

malignant change. These lesions occur on any part of the body, but most frequently on the extremities and exposed parts (Howes and Birnkrant; Butterworth and Klauder). Trauma may have a possible influence on their frequent occurrence in exposed locations (implantation melanoma); according to Taussig and Torrey, however, trauma is not always recorded preceding the onset of melanoma. A relatively rare localization is in the nail bed or nail fold (melanotic whitlow of Hutchinson). Pack and Adair (1939) found that 29 (6.1%) of 477 tumors occurred on the hand, and of these 34.5% were subungual in distribution. They list 13 conditions which must be differentiated from this lesion. Sometimes the lesions may arise on the conjunctiva and other ocular sites (reviewed by Reese, 1938 and 1943). Malignant melanoma may affect the nervous system. In their review of this subject Moersch, Love and Kernohan found 34 such cases (of which 19 were verified at autopsy) in 500 cases of melanoma observed at the Mayo Clinic from 1930-1939. In about one-quarter of the cases, the primary tumor was about the eye. In at least 347 cases of melanoma there was evidence of recurrence or metastasis at the time the patients were seen at the clinic. Most of them died, and in at least 34 of these 347 patients there was clinical evidence of central nervous system involvement. Netherton offers three possible explanations of the relation of the pigmentary changes in the meninges to the pigmentation on the skin: (1) melanoma of the meninges as a metastatic lesion coming either from the nevus or the eye, either being the usual location of primary malignant melanoma; (2) the lesions being of a different type, unrelated and only coincidentally associated; (3) the nevus and changes in the central nervous system being related in that they were congenital malformations resulting from widespread developmental disturbance in the ectoderm. He regards the third as the most plausible.

It is stated that about one-third to two-

thirds of the malignant melanomas arise from a pigmented nevus. This fact is substantiated by most authors (among them Montgomery;¹⁹³⁴ Butterworth and Klauder; Becker, Taussig and Torrey; Howes and Birnkrant and Traub;³⁰⁵ Traub and Keil³⁰⁶). Those who claim they usually arise from normal skin may do so because of faulty observation on the part of the patient to notice a relatively insignificant spot or because the lesion may be occasioned by the presence of a congenital cell rest which remains quiescent until stimulated to active growth. On the other hand, there is disagreement as to the type of nevus which is the precursor of the malignant melanoma. Montgomery (1944) claims that contrary to what has been stated in the literature (Taussig and Torrey; Traub; Traub and Keil), they may arise from both the superficial flat or junction type of nevus and the ordinary hairy mole. We believe that if this latter phenomenon is possible, it must occur very rarely. Lentigo maligna, which is a deeply pigmented flat macule or slightly raised papule may remain as such or result in verruca senilis, senile keratosis or malignant melanoma. All types of transitions between various pigmented spots and nevi and malignant melanoma have been described.

The course of malignant melanoma is variable. It is well described by Butterworth and Klauder, and Montgomery.¹⁹³⁴ The first symptom of malignancy is a very rapid increase in size and the lesion may become verrucous, fungoid or ulcerative. Increase of pigment occurs at the same time or soon thereafter. Montgomery (1944) recognizes fine radiating lines of pigment extending up the lymphatics as indicative of extension. Bleeding also occurs early, but may not appear until some months after increase in size. Metastasis may occur early in the disease and may skip adjacent lymph nodes to show involvement of the internal organs. Multiple cutaneous metastases may be seen and, depending on the rapidity of growth, may be pigmented or not

(amelanotic melanoma). Those from the eye are usually amelanotic. Eller and Schonberg⁸⁵ observed a patient with metastatic melanocarcinoma with apparent recovery. Histologically, this tumor is represented by the presence of malignant nevus cells. Biopsy may be necessary to rule out other blue black nodules which are not malignant melanoma.

Most authorities agree that electrocoagulation or cautery are dangerous and that radiation therapy is to be discouraged. Best results are obtained by radical excision, amputation if necessary, and dissection of lymph nodes, prophylactically (Peck and Adair; Howes and Birnkrant). There is much discussion and disagreement regarding the prophylactic removal of moles, but a safe guide would seem to us to be that since removal of all moles is impractical, those which are subject to repeated trauma, especially at sites of frequent occurrence of malignant melanoma (head and feet) should be removed by wide excision or through destruction as advised by Klauder.¹⁴² Casual handling of this problem leads to what Tod has aptly termed the "tragedy of malignant melanoma."

An interesting note by Herbst¹¹⁸ on "malignant melanoma of the choroid with extensive metastasis treated by removing secreting tissue of the testicles" was followed by a case of failure to cure melanoma by this means. A summary of the facts which link melanoma to the sex glands has been made by Howes.¹²⁷

Connective Tissue Tumors. Keloids. These are benign, proliferative fibrous tissue overgrowths having their origin in the subpapillary plexus of the cutis and developing as a result of trauma. Keloids are fully discussed in the review of Garb and Stone.⁹⁵ Because they have distinctive characteristics, they occasion little difficulty in differential diagnosis from other benign growths. Histologic study is obligatory for final evaluation.

Histiocytoma Cutis. Fibrotic nodules of the skin have been studied recently by

numerous investigators: Sweitzer and Winer;^{289c} Keil;¹⁴⁰ Urbach and Hill;³¹⁰ Michelson;¹⁸⁷ Traub and Monash;³⁰⁷ Senear and Caro;²⁶⁴ Stecker and Robinson;²⁷³ Lewis and Sachs;¹⁵⁷ Bernstein;³¹ Arnold and Tilden.¹⁴ In their paper Sweitzer and Winer discussed the various types of fibrotic nodules which occur in the skin. They included nodules which appeared after trauma, and after certain general systemic diseases, subepidermal fibrosis and knuckle pads. We shall only discuss histiocytoma cutis (subepidermal fibrosis).

Histiocytoma cutis has been designated by a variety of names, including fibroma simplex, nodulus cutaneus, dermatofibroma lenticulare, dermatofibroma, nodular subepidermal fibrosis and by Urbach and Hill, imbibitio lipoidica collageni degenerati cutis. These tumors are of frequent occurrence; they may be single or multiple and are located mostly on the extremities but do not favor the region of joints. The sites of predilection are the extensor surfaces of the tibia and forearms, but any part of the body may be involved. They are small, superficial, hard, flat, asymptomatic lesions which range from about 0.5 to 3 cm. in diameter. Their color varies from red to dull brown, depending on their age. When removed, the lesions show no tendency to recur. Histologically Sweitzer and Winer "observed in all of them a circumscribed proliferation of the collagenous fibrils in the deep reticular cutis. The interfascicular spaces are compressed, obliterating the lymph spaces. Sebaceous glands are absent, and sweat glands below the involved area are atrophic or compressed. Histologically the lesion is stellate and contains few vessels in its substance. The periphery, although not encapsulated, stands sharply in contrast with the normal connective tissue which circumscribes it. At the junction of the normal and abnormal tissues the blood-vessels show marked sclerosis. The elastic tissue within the lesion is greatly diminished or entirely absent. One is impressed, except for the irregularity, by the similar-

ity to a true scar." Michelson thought these lesions were a response of the connective tissue to a low grade excitant which does not bring about suppuration or liquefaction but results rather in scar formation similar to keloid. Sweitzer and Winer, by study of frozen sections using stains for fat and injecting saccharated iron (1%) into lesions, were unable to note any excessive presence of fat or iron granules in phagocytic cells, as mentioned by Senear and Caro. They agree with Stecker and Robinson that the histiocytes reported are probably wandering phagocytes and that the tumor itself is not a histiocytoma. Urbach and Hill likewise rejected the histiocytoma cutis concept for these lesions and thought their evidence favored the classification of these lesions in the category of a new local lipoid dermatosis "imbibitio lipoidica collageni degenerati cutis."

Arnold and Tilden studied 27 lesions and 23 patients. They concluded that histiocytoma cutis is a tumor resulting from sharply localized proliferation of cells which have the ability to, and usually the opportunity to, phagocytose lipids or other foreign material and are therefore of reticulo-endothelial system origin. Cells invade, damage and displace normal dermal collagen instead of producing new collagen. It is related to xanthoma group and not fibroma. Like xanthoma, it is probably related in some way to type of metabolic disturbance observed in diabetes mellitus. Histologic study is needed to differentiate from fibroma. They occur in women and men in a proportion of 5 to 1. Age and race are not important factors. About 50% of the cases had a close relative with diabetes. Of 22 cases, 15 gave a positive reaction for fat to the Sudan III stain. No typical foam cells were found except in 1 lesion. In 7 cases some cells resembled foam cells.

Oil Tumors. In 1920 Mook and Wander²⁰² described the occurrence of painful tender slowly-growing tumors in 6 patients who had been previously subjected to serious operations and had received

camphor in oil injections as a stimulant. Since then numerous studies of the tumor response to the injection of oils have appeared: Stokes;^{278b} Burrows and Johnston;⁴³ Sutton;²⁸¹ Weidman and Jeffries;³²⁶ Jorstad and Glenn;¹³⁸ Burrows and Jorstad;⁴⁴ Rosser and Wallace;²⁵³ Conrad, Conrad, Jr., and Weiss.⁶⁰ These tumors induced by various oils (including liquid petrolatum, linseed oil, corn oil, sesame oil) are not in reality neoplasms, but represent foreign body reactions to the oil.

Hemangio-endothelioma. The designation hemangio-endothelioma (Mallory¹⁷⁶) has been loosely applied to identify tumors showing similar clinical features but with variable microscopic findings. Sweitzer and Winer^{289b} (1936), in their report of 6 cases and review of the literature, defined it as a tumor which may occur on almost any part of the body. Its size may vary from that of a pea to that of an orange. It is usually dark red and moderately soft and may or may not be surrounded by satellites. Some of their patients presented a large tumor. The neoplasm often ulcerates on trauma and bleeds profusely. It infiltrates and can attach itself to underlying tissue and bone or to the overlying skin. According to its site of development, it may at times form a pedunculated tumor. It is not tender and is not painful when manipulated.

Histologic structure: The tumor was considered in 3 related zones, each gradually fusing into the other without any definite line of demarcation. By this schematic method the explanation of the histologic picture is simplified. In the center of the mass are mature structures; progressing toward the periphery, the vessels gradually become more immature. Thus the central core consists of capillaries filled with blood. Surrounding this central core are solid cords or strands of cells which are in the process of forming lumens, while on the extreme outer border or periphery of the tumor are irregular masses of cells showing no features of differentiation. The individual cells are

large and exhibit a clear cytoplasm and a nucleus that is small in comparison with the size of the cell. These cells are endothelial cells but cannot be recognized as such except when an embryologic capillary with a lumen is formed. Naturally, many cases vary in various ways from this description.

Stout^{280c} required the presence of numerous atypical endothelial cells in excess of the number needed to line the vessels with a simple membrane and also the formation of vascular tubes with a delicate framework of reticulin fibers and a marked tendency for their lumens to anastomose. Hemangio-endothelioma is to be differentiated from hemangioma, vascular sarcoma, granuloma pyogenicum tumors and Kaposi's disease.

Caro and Stubenrauch⁵⁰ in their careful report regard malignant vascular tumors as a rarity and think that the literature suggests that a large proportion of such malignant tumors belongs to the group of hemangio-endotheliomas. Diagnosis is not possible on clinical grounds alone, but depends on the demonstration of typical histologic changes (masses of atypical endothelial cells and vascular tubes, lined by several layers of endothelial cells and have a tendency for their tumors to anastomose). These authors produce a convincing summary of evidence indicating that trauma is an important factor in the production of these lesions.

Von Recklinghausen's Disease (Neurofibromatosis). Von Recklinghausen's disease is a process of varied clinical character. Its most frequent and almost constant feature is the presence of multiple cutaneous tumors (molluscum fibrosum), which vary within wide ranges in extent and size. These lesions do not show evidence of localization corresponding to that of the peripheral nerves. In addition, pigmentary disturbances of the skin are numerous and frequently precede the appearance of the tumors. These appear as various sized macules and plaques. There may be tumors of the central and peripheral nervous systems

and of the viscera, associated anomalies of bones and possibly endocrine disturbances. Low standards of physical and mental standards have been reported by various writers (Charpentier;⁵³ Ormsby and Montgomery;²¹⁶ Levin and Behrman).¹⁶⁴ Based on histologic evidence, many authors (summarized by McNairy and Montgomery)¹⁸³ have described the presence of nerve fibers in lesions of the peripheral nerves. The histologic picture of cutaneous tumors is less definite. They are sharply circumscribed and have a structure distinct from that of the adjacent cutis. They are described as being composed of peculiar nucleated bands of pale, fine, spindle shaped fibrillæ resembling those seen in nerve tissue. The absence of elastic tissue has been observed. The presence of nerve fibers has been reported by some authors and denied by others. McNairy and Montgomery have collected the literature on this point. Because of the uncertainties regarding the presence of nerves in these cutaneous tumors, McNairy and Montgomery reported on a histologic study of the problem based on 15 cases and on a search for nerve fibers by Bodian's method of staining.¹⁸³ In 12 cases, non-medullated nerve fibers were clearly demonstrated. This supports the thesis that these lesions are true neurofibromata which arise from the peripheral nerves and their supporting structures and that these nerve fibers are peripheral extensions of the nerve bundles which have been included in the expanding nerve sheath tumors. The cutaneous tumors of von Recklinghausen's disease thus may be said to have a specific histologic architecture and are thus readily distinguished from other cutaneous tumors or from the tumor-like macular atrophy of Schweninger and Buzzi. Pigmented macular lesions (2 studied) showed no histologic evidence of an underlying neurofibromatous structure or of an increase in nerve fibers.

Thannhauser²²⁴ recently presented an exhaustive study and revaluation of the apparent relations of neurofibromatosis

(von Recklinghausen) and osteitis fibrosa cystica. On the basis of reports of simultaneous occurrence of nodular cutaneous neurofibroma, pigmented areas of the skin and osteitis fibrosa cystica in a single patient, a relationship between neurofibromatosis and osteitis fibrosa cystica is suggested, since the occurrence of these symptoms of 2 rare diseases in 1 person cannot be mere coincidence, according to Thannhauser. Fibrocystic involvement of localized areas of the skeleton, especially the long bones, occurs in neurofibromatosis together with *café au lait* spots and cutaneous neurofibromas. Although microscopic examination of the osseous fibroma does not reveal nerve structures within the fibroma, whorls of spindle cells are occasionally found and may be thought to reflect its neurofibromatous origin. Thannhauser believes that hyperpigmented areas are the expression of underlying neurofibromatosis even without the appearance of neurofibromatous nodules. The presence of similar endocrine symptoms, especially the concurrence of precocious puberty and pigmented blotches of the skin in neurofibromatosis and also in osteitis fibrosa cystica disseminata offers additional corroboration of the belief that the 2 disturbances are related. This suggests that the anatomic structures which underlie the endocrine symptoms in cases of simple neurofibromatosis are the same as those of osteitis fibrosa cystica disseminata (von Recklinghausen).

Cutaneous Neuroma. This exceedingly rare lesion has been the subject of study by Ludy¹⁶⁹ and by Montgomery and O'Leary.²⁰⁰ This type of tumor is a lesion which contains abnormal growth nerve elements and has its location and development in the true skin. They are distinct from the nevi containing lesions of von Recklinghausen's disease. The nodules are painful and may be incorrectly diagnosed as some granuloma such as tuberculosis clinically. Montgomery and O'Leary carefully reviewed the literature on this subject and described a heretofore unrecognized process which they called

multiple ganglioneuroma of the skin. The disease consisted of discrete, firm, yellowish papules or nodules of from 1 mm. to 1 cm. in diameter. During their development the reddish brown color of the lesions suggested sarcoid or tubercloid. The lesions first appeared on the buttocks, later spread to the trunk and extremities. The healing of lesions was followed by the development of new lesions. The entire disease had left the patient without a trace within 3 years. Histologically the condition was characterized by the presence of numerous large pale staining ganglion cells.

Kaposi's Disease (Multiple Idiopathic Hemorrhagic Sarcoma). In 1872 Kaposi described the process now generally called Kaposi's sarcoma which he called multiple idiopathic pigmented sarcoma at first, and later multiple idiopathic hemorrhagic sarcoma. Excellent recent publications dealing with this subject are those of Dörffel;⁷⁶ MacKee and Cipollaro;^{172a} Becker and Thatcher;²⁴ Symmers;²⁹⁰ Aegerter and Peale;⁶ Nesbitt, Mark and Zimmerman;²⁰⁵ Sachs and Gray.²³⁶

A concise description of the lesions of this disease is given by Aegerter and Peale⁶ as follows:

"The disease is most commonly primary in the skin, though the primary process has been reported in almost all parts of the body. It was the cutaneous type which Kaposi described. The lesion usually begins as a reddened macular area on an extremity, most commonly a lower extremity. The edges are usually definite but there is no characteristic shape. The macule varies greatly in size, depending on how long it has been present. As the lesion progresses, the color of the involved area darkens, becoming first dusky red, then bluish and finally brown or black. This change of color is understandable after microscopic study of the lesion. At first there is an increase in the supply of blood to the part through the cavernous vessels. At this time the area is livid. But as the cellular elements of the tumor increase, there is stagnation of the blood, and finally hemorrhage with rather remarkable pigmen-

tion, which probably results from the disintegration of the blood. The involved area soon becomes elevated and in the early stages resembles a varicosity. As the tumor ages, becoming more cellular and less vascular, it becomes firm, and its appearance is that of a true neoplasm. The lesion rarely regresses spontaneously, although it may remain stationary for months. Healthy areas of skin proximal to the lesion may soon become involved. The older lesion may fade out, become keratinized and present a scaly surface. The subcutaneous tissues become infiltrated, and the lymphatics are frequently blocked, causing an immense edema, even elephantiasis, of an extremity. Sometimes the lesions are pruritic, and trauma may cause them to bleed freely. Sooner or later, lesions usually appear in the viscera. Some writers believe these to be spontaneous multiple primary growths because they are able to find all the stages of development in these lesions. If we accept the opinion that this disease is cancer from the outset, and such is our premise, we may regard these secondary lesions as metastases, following the usual course of cancer. Death may occur from infiltration of the lungs, the liver or the spleen but by far the most common sequence is metastasis to the intestinal mucosa with ulceration causing hemorrhage and exsanguination. When the tumor is primary in the viscera, it is usually not found in the skin. . . . Metastasis to lymph nodes is not general, but it occurs, and the metastatic lesions reproduce the primary cellular picture.

"If sections are made of the very early lesions in the skin, the microscopic picture is that of cavernous hemangioma. The sinuses vary in size and shape, they contain blood and they are lined by normal appearing endothelial cells. No perithelial cells could be distinguished in any of our slides. As a lesion progresses, it is seen to penetrate into the surrounding normal tissues, splitting muscle bundles and infiltrating the fat. This is seen in the very early lesions. The sinuses are less perfectly formed at this stage, and they are surrounded by masses of fusiform cells without pattern, the nuclei of which are indistinguishable from those of the endothelial lining cells. A little later the sinuses become indistinct and are represented by spaces which are filled with blood.

There are hemorrhage and considerable red cell degeneration, as shown by the quantities of pigment which take the iron stain. The endothelial lining is now almost indistinguishable, and there is an apparent transition of the endothelial into the fusiform cells. The latter cells proliferate to form masses, some of which are quite avascular. Sections through these areas are easily confused with fibrosarcoma or even neurogenic sarcoma. These solid masses occupy the corium, pushing upward to flatten the papillæ, causing thinning of the epidermis. It is these masses that give rise to the nodular gross appearance. By this time there is usually an inflammatory reaction, as shown by an infiltration of lymphocytes, plasma cells and a few macrophages. This may be accounted for by the hemorrhages. Differential stains give the staining reaction of young fibrous tissues. In some tumors these cells show definite malignant characteristics. In some instances the solid cell masses have the appearance of endothelioma. The cells are polyhedral and arranged in sheets about irregular sinuses, some of which contain erythrocytes.

"The duration of the disease varies from 8 months to 25 years although in the majority of instances the process terminates after 5 to 10 years. In a few cases it has been reported to have regressed, with the patient remaining free of symptoms for a number of years. We are tempted to question the diagnosis in these cases since by far in the majority of over 600 reported cases the disease was progressive, though sometimes remittent in character."

The nature of this disease has been considered to be sarcoma, granuloma of nerve tissue or a blood-vessel and lymph vessel disorder of the nature of angioma, hamartoma, connective tissue proliferation with vascular dilatation, proliferation of sympathetic perivascular nerve fibers, or reticulo-endothelial system disease with a disturbance in its monocytogenic function. Becker thought that the most logical explanation of Kaposi's sarcoma was that it is a multicentric benign neoplasm originating in the perithelial tissue from embryonic mesenchymal cells (lymphocytoid cells of Marchand), which results in a type of cell growth unique for

the disease. Aegerter and Peale thought it was a vascular cancer. Symmers thought that the unit of growth in the cutaneous nodules of Kaposi's disease is the fibroblast.

Becker lists 36 synonyms for the disease. The cause of the process is unknown. It is presumed to be a toxic or infectious substance which acts on the vascular system with resultant inflammatory reaction, vascular dilatation and subsequent proliferation of the vascular wall elements. Dillard and Weidman⁷³ found fungi resembling *Achorion schoenleinii* in the lesion, but did not believe them to be pathogenic. Greco¹⁰⁵ found an organism of the cryptococcus type. The relationship of this to the origin of the disease is unproven. The disease occurs chiefly in Italians and Hebrews. Ellis⁸⁶ reported a case in an American Negro. It occurs chiefly in persons between 50 and 70 years of age. Men are the chief sufferers. In addition to the cutaneous lesions there may be lesions in the gastro-intestinal tract, liver, lungs, abdominal lymph nodes, spleen, pancreas, kidneys, suprarenals, testes, epididymis, trachea, thyroid, bronchi, pleura and heart muscle. The nervous system is rarely affected but Nesbitt and his co-workers reported lesions in the brain.

Nevi. Nevi are, in the broad sense, any congenital circumscribed new growth, but the term is commonly used as synonymous for ordinary cellular nevus (mole). In a learned paper Meirowsky¹⁸⁴ discusses the inheritance of nevi and other malformations of the skin; he concludes that man has become prone to nevi and malformations of the skin by the factors both of domestication and phylogenesis. A convenient classification of the various types of nevi is that of Traub:¹⁶⁵

I. VASCULAR NEVI.

A. *Nervus vasculosus* (hyperplasia of blood-vessels).

1. Flat (capillary). Example: nevus araneus of nevus flammeus.
2. Raised (capillary). Example: angioma.

I. VASCULAR NEVI.—(Continued).

3. Deep (capillary). Example: cavernous angioma.

B. *Nevus lymphangiectodes*.

C. *Nevus vasculosus et lymphangiectodes*.

D. *Heterogeneous vascular nevus*. Example: combination of the above types in the same mark.

II. PIGMENTED, HAIRY AND/OR WARTY NEVI.

A. *Intra-epidermal nevus* (no nevus cells). May terminate in basal cell or squamous cell epithelioma. Possible examples:

1. *Nevus verrucosus* (hard). May also present some hairs or pigmentation, and frequently has a linear configuration.

2. *Verruca senilis* or *keratoma senile*. Generally becomes basal cell epithelioma if a change takes place at all.

3. *Dermatosis papulosa nigra*.

B. *Epidermo-dermal nevus* (junction type nevus). Nevus cells present. May terminate in melanoma (nevocarcinoma). Example: *Nevus pigmentosus*. May rarely also present a few fine, or *sparse* coarse hairs or have a *soft* warty surface.

C. *Intradermal nevus* (nevus cells present). Benign always, if pure type. Example: *Nevus pigmentosus et verrucosus et pilosus*. Always soft and warty in character—not hard.

D. *Blue nevus* (cells of different shape than in preceding types). May rarely terminate as melanosarcoma.

E. *Heterogeneous pigmented nevus* or combination or mixture of two or more of the above types (nevus cells present). May terminate as basal cell or squamous cell epithelioma, as melanoma or melanosarcoma.

III. *Nevi of Special Tissue*.

A. *Glandular* (sudoriparous gland nevus, sebaceous nevus).

B. *Connective tissue*.

C. *Fat tissue*—nevus lipomatodes.

D. *Nerve nevus*.

E. *Heterogeneous type*.

Vascular Nevi. Vascular nevi have been discussed in a number of papers (Lister;¹⁶¹ Vohwinkel;³¹⁴ Touraine and Bupperat;³⁰³ Oughterson and Tennant.²²⁰ The reader is referred for a complete discussion of the question of spider angioma (*Nævus araneus*) to the excellent monograph by Bean.²²

Pigmented Hairy and/or Warty Nevi. Traub in 1941 described in some detail the clinical features of the nevi mentioned in his classification. The intra-epidermal nevus consists of lesions that lie entirely within the epidermis and are formed by proliferation of a mixture of normal prickle and basal cell type epithelial cells. There are no nevus cells. Clinically the lesions appear to be superficial, contain a variable amount of pigment, and may assume a linear configuration. They rarely terminate in cancer. Evidently Traub is using this term for what we commonly speak of as seborrheic verruca or senile keratosis. Waisman and Montgomery,³²⁹ in their discussion of verruca plana and epithelial nevus, used the latter term for verrucous and papillomatous nevi which were first systematically classified by Unna, under the designation "hard nevi," in which group he included circumscribed warty protuberances with the microscopic appearance of purely epithelial thickening. Unna described the lesions as being clinically hard, flat, pink to gray or dark brown and of variable size, from that of a lentil to that of a pea, the surface being rough or finely granular. Histologically, he distinguished 3 types, determined by the site of epithelial proliferation, as the keratoid, the acanthoid and the mixed or combination form. Of Unna's 3 types, 1 or possibly 2 were verruca senilis, a lesion which may be a delayed epithelial nevus with distinctive histologic picture. Examination of certain epithelial nevi, of both the circumscribed and the linear form, indicates that pathologic changes may be present which are practically identical with those present in epidermodysplasia verruciformis. The vacuolar protoplas-

mic abnormality which each form manifests indicates the essential nevoid structure common to them. Under the designation "mole," Traub regards a cellular nevus characterized histologically by the intracutaneous arrangement of nevus cells in special configurations (nests, bands and strands). Nearly all such lesions show the presence of a variable amount of melanin pigment both clinically and histologically, but this item is not obligatory. These growths may be present at birth or appear shortly after birth or later in life. From a clinical standpoint there are all sorts of transitions between various types of nevi. This complicates the problem because it brings up the question of possible transitions in type between the epidermodermal and intradermal nevi. The junction type clinically (Traub) "is the relatively flat or slightly raised smooth pigmented mark that is devoid of coarse hairs. Hairs are usually entirely absent, but fine (lanugo) hairs or occasional coarse sparse hairs may be observed both clinically and histologically. As a rule, the hairs are never a striking feature, as most of these marks are described as devoid of hairs. The surface is usually smooth, but as the lesions get older or take on added growth, they may develop an irregular or even soft verrucous or fungating surface. The size may vary from a tiny mark no larger than a pinhead to one of considerable dimension, but the largest marks that I have so far encountered have not been over 3 to 4 cm. in diameter and the majority do not exceed 1 to 3 cm. in diameter. They are found most commonly on the extremities and the face—less frequently on the trunk—but they may occur anywhere. On the trunk the most frequent location seems to be the upper back (shoulders) and the next the abdomen."

Traub and Keil²⁰ define the histologic features of the "common mole" as:

"1. Intra-epidermal nevi lie entirely within the epidermis and present a proliferation of normal epithelial cells, a mixture of

prickle and basal cell type, not referred to as nevus cells. They may or may not be pigmented. They rarely terminate in cancer.

"2. Intradermal nevi are probably the most frequently encountered lesions and are characterized by the occurrence of nevus cells arranged intracutaneously in nests and strands. The cells are of the mature type, and to become malignant they revert to the embryonic type. Pigment is generally present in varying amounts. Cancer of malignant melanoma rarely, if ever, occurs.

"3. In junction type nevus (Satenstein) or borderline melanoma Becker stated: 'The process may be interpreted as hyperpigmentary due to an increased number of melanoblasts at the epidermo-dermal junction.' The cells are not separated from the epidermis, as in the intradermal nevi, in which the nevus cells are seen in nests or strands usually detached from the epidermis. The cells in this type are of the embryonic (anaplastic) type, and the lesion constitutes the forerunner of the malignant melanoma. The criteria necessary to decide whether a junction nevus has definitely become a malignant melanoma require careful consideration of both the clinical picture and the course of the lesion, as well as a minute study of the histologic changes. Lesions are encountered which clinically seem innocent and which apparently pursued a benign course as long as we have been able to keep the patient under observation. But the reports on slides studied in these cases by various pathologists show great differences of opinion. If a report of malignant melanoma had been accepted in such cases without due consideration of the innocent clinical course, great harm might have been done. We believe that the clinical criteria for malignant melanoma have been sufficiently stressed so that it would be superfluous for us to restate them, but the histologic grounds or basis for such a diagnosis certainly seem less clearcut.

"It appears logical that the following points are necessary for the diagnosis of malignant melanoma: (1) 'trickling off' and segregation (development of théques) (one of the principal changes and found without difficulty), (2) the presence of clear cells, which may resemble the Paget cells, (3) mitotic figures (generally readily found), (4) chronic inflammation. The more evident each of these changes appears in the

section, the more certain one may be of the malignant nature of the growth.

"4. The combination type nevus represents a combination of Types 1, 2 and 3.

"5. The blue nevi (slate blue or grayish blue), which are chiefly found as isolated lesions on the face and dorsum of the hands, have no relation to melanoma. The color is apparently determined by the number and depth of the melanoblasts. It must be emphasized that the name blue nevus should be restricted to pigmented growths composed of a peculiar type of connective tissue cells apparently capable of forming melanin; that so far as the literature and our limited observations are concerned these spots are benign in their course, that that diagnosis on the basis of color alone is unreliable, as other lesions, such as the melanoma and the 'common mole,' may reveal similar shades of blue. In rare instances an ordinary intradermal mole may show clinical features apparently indistinguishable from those of the blue nevus; this phenomenon is apt to occur when the nests of nevus cells and their accompanying pigment are separated from the epidermis by a wide stretch of intervening connective tissue. It is interesting that a number of cases have been recorded in which the characteristics of an ordinary mole were associated more or less intimately with those of the blue nevus of Tietze. This represents another example of a combination of nevi. It has been claimed that blue nevi may terminate in sarcoma, but such a change must be rare indeed, for Satenstein, Becker, Peck, Ewing, Wood and Fraser have not seen examples of it." (Blue nevus will be discussed below.)

Montgomery and Kernohan¹⁹⁸ made an intensive study of pigmented nevi. The nevus cell of the common ordinary pigmented nevus has been maintained by various authors to originate from single and from multiple sources. Origin from basal or dendritic melanoblastic cells in the epidermis (Kissmeyer; Miescher; Perin), mesodermal origin from connective tissue cells of various types, including also origin from the adventitia of the blood-vessels (Stoeckenius), origin from the lymphatic endothelium, multiple neuro-epithelial points or origin from "celules claires" in the epidermis, cells of Schwann

sheaths, neuroid tubes and true nerves in the deeper part of the cutis (Foot; Masson; as well as from peri-endo-neural endothelium of the skin (Feyrter) have been included. Close relationship, if not even origin, of nevus cells from the walls of hair follicles, from sebaceous glands and even from the endothelial cells of the intima of the blood-vessels have all been described (Ebert; Gans; John; McCarthy). Since Masson's articles an increasing number of pathologists have tended to accept multiple neuro-epithelial points of origin for the nevus cell (Becker; Ewing; Foot).

Recently John described a "drop" cell in the middle layers of the epidermis which he called a stalagmocyte. He distinguished this from the Langerhans cell and the dendritic cell and assumed that stalagmocytes play an important rôle in the formation of nevi. Multiple and varied clinical and histologic classifications of different types of true nevi are given in recent articles in the literature, most of which also include extensive reviews of Masson's theory and other theories regarding the origin of nevi (Becker; Broders; Ebert; Foot; Laidlaw; Traub; Zeisler).

Montgomery and Kernohan studied a group of 460 nevi in which ordinary nevus cells (histologically) with special reference to the neuro-epithelial origin of the nevus cell as advocated by Masson and also in regard to diagnosis, prognosis and treatment. Various special nerve and trichrome stains and also stains for pigment reticulum fibers, etc., were employed for many of the nevi. In only 11% was there evidence of *lamcs foliacées*. Origin of nevus cells from the epidermis was demonstrated in a majority of cases. In many instances the nevi apparently arose from cellules claires, but in others from basal or dendritic cells. The question is raised as to whether cellules claires may not represent altered forms of basal or dendritic cells. The histologic evidence would support Unna's older conception of epidermal origin against Masson's con-

ception of neuro-epithelial origin of the nevus cell. Cellules claires can be identified readily in formalin-fixed sections even with hematoxylin and eosin stain but better with Masson trichrome stain. Distinction between fine strands of melanin pigment in dendritic processes of melanoblastic cells and true nerve fibers in the epidermis may be exceedingly difficult as silver stains all stain melanin pigment.

The many cases of superficial flat nevi and lentigines in which histologically nevus cells are present and in which there is no increase in cutaneous nerves or nevus cells deeper in the cutis, would tend to refute a neuro-epithelial origin for the nevi. This is especially true if the cellules claires are regarded as melanoblastic cells rather than modified tactile cells of Merkel-Ranvier. On the basis of our studies we are strongly inclined to support Unna's old concept of an epidermal origin for the majority of the nevi, admitting a nerve origin for about 15% of the cases in which *lames foliacées* are present. Just as we see multiple types of nevi in the same individual, so multiple points of origin for the nevus cell might be expected, inasmuch as we are dealing essentially with a malformation. In our series of 460 nevi, 1 instance of apparent origin of nevus cell from sebaceous cell was seen and in several instances, nevus cells apparently were intimately connected with, if they did not arise from, the endothelium of the capillaries of a hemangioma.

The question of malignant change in pigmented nevi merits discussion. Superficial nevi (so-called junction nevi) in which collections of malignant-looking nevus cells are limited to the upper portions of the cutis or occur as intra-epidermal nests (*thèques*) and in which the condition is suspected histologically of being early melano-epithelioma usually offer a good prognosis. These nevi should be distinguished from cases of frank melano-epithelioma in which the prognosis is almost invariably serious, at least

almost invariably so when there is histologic evidence of invasion of the lymphatics by the tumor cells.

Pigmented nevi should not be confused with blue nevus or with verruca senilis. Pigmented nevi, whether superficial or deep, papillomatous or verrucous, or slightly to deeply pigmented, need not be removed except for cosmetic purposes unless they are subject to repeated trauma and irritation. Surgical excision is the method of choice. Partial removal of superficial fulguration, electrolysis, the use of solid carbon dioxide or caustics, although apparently satisfactory in the hands of many dermatologists, in our experience have the potential danger of subsequent development of malignant epithelioma in a small percentage of the cases. Radium and Roentgen therapy in any form is to be condemned vigorously.

Finally, as to nevi, Montgomery and Kernohan have stated that the nevus cell probably has multiple points of origin but that origin from the epidermis, as Unna originally emphasized, predominates. Evidence of a neuro-epithelial origin with the presence of *lames foliacées* occurs in 10 to 15 or possibly as high as 20% of the nevi. Origin from the outer walls of hair follicles may be anticipated in view of the predominant epidermal origin. The rare instance of origin of nevus cells from sebaceous glands and an occasional intimate relationship of nevus cells to the endothelial cells of the capillaries are illustrated in this article.

Ebert⁷⁸ suggested that the cellular type of nevus is formed from many elements, blood-vessels, histiocytes, connective tissue cells, nerve fibers and various other cells. The nevus cell is the unique element, and a study of its origin has been the primary object of Ebert's study. It is the parent cell of the malignant melanoma. Greenblatt, Pund and Bernard¹¹⁰ present a schematic outline of the histologic findings in the gradual transition of the benign nevus to the malignant melanoma. Arnold¹¹³ reported a case of multi-

ple tardive pigmented nevi, unusual clinically in that the apparently mature lesions showed a tendency to central papule formation, and histologically in that these same seemingly mature lesions showed only beginning extension of nests of nevus cells in the corium.

The Blue Nevus. The blue nevus was first identified by Tiéche²⁹⁸ in 1906. This is a fairly frequently seen lesion in spite of the relative inattention which has been accorded it. For example, Becker^{23a} does not mention it in his valuable paper on melanotic neoplasms of the skin. Undoubtedly it has been mistaken for early malignant melanoma because of the slate black color. It remained for Montgomery and Kahler¹⁹⁷ to describe completely the process for the American literature and for Traub³⁰⁵ to further indicate the relative position of this benign growth among the various types of pigmented and hairy nevi. For details and for a summary of the literature the reader is referred to these papers; but the essential facts regarding it have been summarized by Montgomery^{193a} as follows (see also above):

"BLUE NEVUS. I believe blue nevus to be a relatively common disease as I have encountered more than 100 typical cases. I believe that in the past it has been confused with melano-epithelioma and nevus pigmentosus. Blue nevus is a sharply circumscribed, smooth, round or oval, indurated papule or nodule usually varying from 2 to 15 mm. diameter, occurring most frequently on the face and hands but to be found anywhere on the body. The color varies from dark or mottled blue to blue black, blue gray or even steel blue. There usually is a history of a solitary lesion which either has been present since birth or developed in infancy or early childhood and which has remained as such without increase of size. Such a history is diagnostic. Two or more lesions sometimes occur in the same case. Occasionally, blue nevi develop later in life, and, when they are steel blue and have increased in size, they may be indistinguishable clinically from a melano-epithelioma. Wide excision and histopathologic examination then are indicated.

"Blue nevus is characterized histopathologically by well-formed, mature, dermal, dopa positive melanoblasts having long bipolar dendritic processes laden with melanin pigment. Such melanoblasts are found in smaller numbers in so-called Mongolian spot. Mongolian spot is much larger than blue nevus and occurs as an ill-defined, bluish to mulberry colored or blackish plaque, usually in the vicinity of the sacrum. It is present at birth but usually disappears in the third or fourth year of life. Blue nevus is a benign lesion and only exceptionally, following repeated trauma and inflammation, does it undergo malignant change. In such cases it forms a true melanosarcoma rather than a melano-epithelioma."

Connective Tissue Nevus. Steiner²⁷⁴ in a scholarly article reviews the knowledge of this rare nevus. It is a lesion consisting of papules or lichenoid nodules varying in size from a pinhead to that of a millet seed, and in color from snow white to brownish. The nevi are usually single. They are usually located on the chest, usually the upper third. In almost all cases, the nevi attracted attention because of their systemization, usually in bands, sometimes in zosteriform arrangement. They are usually asymptomatic. Histologically there are epithelial changes in some of the cases; but the characteristic, and usually the only, changes are in the cutis. All authors agree on the fact that there are changes, but there is disagreement as to the degree of involvement of the collagen or the elastic tissue. Steiner states that the changes are usually homogenization and hypertrophy and sometimes apparently an altered arrangement of the collagenous tissue. There is elastorrhexis, elastoclasia and atrophy of the elastica. Rarely there are atrophic changes of the collagen fibers. Collagenous and elastic tissue alterations can be observed in the same case or independently of one another. The changes are mostly in the upper cutis. These cases must be differentiated, especially from lichen albus, scleroderma in bands, white spot disease and post-zosteriform scars. Pseudoxanthoma elasti-

cum may be difficult to differentiate from fibrous nevus.

Nevus Unius Lateris. Linear nevus, an interesting type of nevus known by a variety of names, has been the subject of numerous investigations. Pack and Sunderland²²² who have collated the important literature on the subject describe it as "a papillary or verrucous congenital tumor having a unilateral or nearly unilateral distribution in linear streaks or bands following the long axis of the limbs or extending transversely around the trunk. This unique disease is so striking that it is readily recognized as a distinct clinico-pathologic entity." It is rare, these authors having found 4 cases among 20,000 patients with tumors studied at the New York Memorial Hospital for Cancer and Allied Diseases (0.01 %).

The cause and pathogenesis of nevus unius lateris are still unsatisfactorily explained. The relationship of this process to the nervous system is still an unsettled question. It is probable from Pack and Sunderland's work that the primary involvement is in the epidermis rather than the dermis. The matter of malignant degeneration of nevus unius lateris needs further study.

A variety of unilateral nevus especially interesting to dermatologists is nevus unilateris comedonicus (nevus follicularis keratosus of White), which Sweitzer and Winer studied.^{289a} It is characterized by comedones unassociated with acne vulgaris, occurring in bands or groups. They are unilateral and in some cases accompanied by suppuration and scarring. Sweitzer and Winer did not feel that this was a variety of sebaceous gland nevus. Histologically, this dermatosis shows markedly dilated follicles filled with a keratinized material with many cysts having their origin in the follicular wall. We have just encountered an exquisite example of this rare type of nevus, in a Negress, extending in the midline from the umbilicus to the pubis.

Nero-xantho-endothelioma. This lesion has been ably discussed in a well-docu-

mented paper by Lamb and Lain.¹⁴⁹ Adamson⁶ in 1905 reported a case before the Dermatological Society of London which was classified as "congenital xanthoma multiplex" in a child of 2½ years. The lesions were for the most part yellow in color, although some had a decided reddish tinge. One on the neck had the appearance of vascular nevus, but on pressure showed a yellow base. Adamson also referred to a case of xanthoma multiplex developing from vascular pigmented nevi reported in 1888 by Kabner.

McDonaugh in 1909 and 1912 described multiple growths of the skin seen at birth or in early childhood which are characterized by their red angiomatous appearance at first, later developing a distinct yellow color and which tend toward a spontaneous involution. They are nevi. Since primary tumor cell in histological studies is related to those cells which form the capillaries and because of the finding of the peculiar endothelial giant cells, he suggests that the tumor be called an endothelioma, though it is not a true endothelioma.

Cases were reported by: Wise; Jacobi and Grand; Goldsmith; Dowling; McGraw; Senear and Caro; Montgomery. Lamb and Lain report a case and conclude that nevo-xantho-endothelioma, while not an accurate term, is fairly descriptive of a congenital malformation in which one encounters peculiar endothelial giant cells, as well as xanthoma cells, histiocytes and fibroblasts, with clinical spontaneous involution of the lesions in later childhood. Lamb and Lain's case is peculiar because of apparent systemic involvement without exophthalmus, diabetes insipidus or membranous bone manifestations. There is a link between cutaneous xanthoma and Hand-Schüller-Christian's disease. The embryonal cell rest theory for formation of this type of xanthomata is stressed by Lamb and Lain.

Osteoma Cutis. Various known as osteoma cutis, osteosis cutis, osteomatosis cutis, chondro-osteoma, miliary osteoma and various other designations, this tumor

is regarded by Vero, Machacek and Bartlett¹³ in their scholarly paper as one of the rare developmental abnormalities of the skin: Osteomas are benign new growths composed of bone and osseous tissue; they occur in most organs, including the skin. Secondary osseous changes may occur in certain cases of syphilis, in nevus, in response to trauma, in scleroderma, in acne vulgaris¹²¹ and in multiple sebaceous cysts;¹²² but the spontaneous (primary) variety is rare. Clinical diagnosis is rarely made without biopsy, but Vero and his co-workers suggest that roentgenograms may be of aid in the diagnosis, as they may detect lesions which might otherwise escape inspection and palpation. These lesions appear in both sexes, especially on the scalp, forehead, cheeks and chin. They may be solitary or multiple. The color, size and shape give no real clue to the nature of these lesions. It is only when bone particles spontaneously extrude or histologic or Roentgen ray study is made that this true nature can be identified.

Mesenchymoma. Tauber, Goldman and Barrett²⁹¹ described a case of a new type of primary tumor of the scalp. It was highly invasive locally and gave rise to metastasis in the ribs and kidneys. It was not an ordinary tumor, and, because the tumor cells were so young and so undifferentiated, the term "mesenchymoma" was applied.

Leiomyoma. These tumors have occasioned much interest in the past. For example, Stokes^{278c} in 1923 described a case of nevoid hyperplasia of non-striated muscle of arrectores pilorum associated with nevus pilaris. Stout^{286b} reported 15 cases (7 in males) of solitary cutaneous and subcutaneous leiomyoma; none were diagnosed prior to biopsy. The origin of the smooth muscle in these tumors is the arrectores pilorum, the muscular apparatus in the depths of the skin or the muscles of the blood-vessel walls. They are of small size and grow slowly. Unlike the multiple form (mostly in males) the solitary form occurs equally in both sexes.

More than half originate in middle or late life. The extensor surfaces of the extremities, the scrotum, labia, nipples or cheeks are the commonest sites. They are painless at first, but as they grow they become painful and tender. The pain is spontaneous or may be favored by fatigue, exposure or trauma. Contractions of smooth muscle in the tumor may result in pain. Nerve fibers have been demonstrated by Cajal's method. Most of the tumors are vascular or angiomatic, although the vessel network is never rich. Only 5 of 70 reported cases showed malignant tendencies. The tumor is elevated, never pedunculated and does not discolor the skin. Diagnosis depends on histologic study. Surgical excision is the treatment of choice, but Roentgen ray, electrocoagulation or cautery have been successful.

Myoblastoma (Granular Cell Myoblastoma). While not generally considered among cutaneous tumors, myoblastoma—a tumor composed of immature skeletal muscle elements—has some interest to the dermatologist because of its resemblance to xanthoma clinically and to carcinoma of the tongue histologically. The first report of such tumors was that of Abrikossoff² in 1926. Since then a number of reports have appeared and the more recent of Klemperer,¹⁴³ of Horn and Stout,¹²⁶ and of Crane and Tremblay⁶⁶ are worthy of study.

Clinically these tumors are small (1 to 2 cm. average), sharply circumscribed or encapsulated. They are benign but some may be poorly demarcated and irregularly penetrate the underlying tissues. Some of the lesions may present ulceration, some hyperkeratosis and others malignant epidermal proliferation. The lesions are usually solitary. Microscopically, the tumors are characterized by large polyhedral cells, the majority being irregularly rounded, but transitional forms having a tear-drop or spindle shape may also be present. The most characteristic feature of the cells is the cytoplasm which is abundant, acidophilic and granu-

lar, not vacuolated. Lipoid stains give negative reactions. Longitudinal and cross-striations have been demonstrated in some cases. The nuclei are small and vesicular, centrally placed, round or oval and associated with numerous pyknotic forms. An interesting feature is the hypertrophy of the overlying epidermis noted originally by Keynes (*Brit. J. Surg.*, 13, 570, 1926) and emphasized among others by Klemperer. They occur at all ages and with equal frequency among males and females. About half the cases occur in persons less than 50 years of age. They are widely distributed over the body but especially in the tongue and mouth. They may occur at sites containing skeletal muscle as well as those free from this tissue. Excision usually effects cure. The nature of the tumor is unknown but they are probably myoblastic or degenerative.

Glomus Tumor. The first case of this interesting lesion was described in 1878 by Kolaczek,¹¹⁶ who called it angiosarcoma. Masson's original description of the glomus tumor in 1924¹⁷⁹ and Popoff's²³⁵ subsequent careful studies on the distribution and alteration of the normal cutaneous glomus in peripheral vascular bed lesions stimulated interest in this subject. The first cases were reported in the American literature in 1934 by Mason and Weil,¹⁷⁸ and by Adair,³ and by a Japanese in 1934 (Aisu⁷). This tumor has been variously called: angioneuroma, angiosarcoma, glomus tumoralis subungualis, Popoff tumors, tumors glomique, tumeur du glomus, neuromyo-artériel, subcutaneous painful tubercle, angiomyoneurome, angiomyo-neuroma of Masson, subcutaneous glomal tumor, angioma perithelioma, false neuroma, glomangiomas, and neuromyo-arterial glomus. Good reviews of this subject which have recently appeared are those of Raisman and Mayer;²⁴⁵ Stout;²⁵⁰ Burman and Gold;⁴² Bailey;⁴⁷ Lewis and Geschickter;¹⁸² Cole and Sroub;²⁷ Jirka and Scuderi;¹³⁵ Hval and Nelson;¹⁷¹ Theis;²³⁷ Weidman and Wise;²²⁷ Bergstrand;²³ Freudenthal, Ander-

son and Weber;⁹³ Slepyan;^{269a} Radasch;²⁴⁴ Grauer and Burt;¹⁰⁶ Davies, Hellier and Klaber;⁷⁰ Ostrowski;²¹⁹ Slepyan;^{269b} and Love.¹⁶⁶

Glomus tumor is not common. Streitmann²⁸¹ was able to collect (1933) only 21 cases which could be identified with this lesion definitely. Lewis and Geschickter report dealt with 17 cases from Johns Hopkins Hospital since 1924. Mason and Weil collected 34 cases; Adair, 10 cases; Stout, 11 cases; Bailey, 7 cases; Jirka and Scuderi, 70 cases, from the literature including their case (1 case in 7000 surgical specimens at Cook County Hospital); and various authors, 1 or 2 cases. We have encountered 5 cases among 2500 biopsy specimens from cutaneous lesions studied in the Hospital of the University of Pennsylvania (1933-45). Although most parts of the skin may be affected, most of the tumors are located in the extremities and generally beneath the nails of the fingers and toes (more than 50%, Bailey). They may occur on the genitalia of either sex (Grauer and Burt).

In about half of the tumors reported there was a previous history of trauma. The average duration of the lesions from the time of their appearance to the development of symptoms was 9 years. The tumors vary in size from 0.3 to more than 2 cm. in diameter. The smaller lesions are usually subungual, while the larger are present in easily distensible tissue. The variable size and pain depend at times on whether or not the tumors are under pressure. Most of the cases present a history of excruciatingly painful crises with radiation of the pain. All the subungual tumors are painful and slight brushing of the affected finger by the clothes or other slight pressure would be sufficient to produce a painful crisis. Scraping of the nail to great thinness was found to reduce the amount of pressure and diminish the pain (Raisman and Mayer). Tumors located in the soft tissues, such as the arms, legs and genitals, are painless. The pain is probably due to pressure and not entirely to a dis-

turbance of the sympathetic nerves since periarterial sympathectomy did not give relief (Burman and Gold).

Although the majority of the tumors occur singly, Weidman, Bergstrand, Stout and others reported cases of multiple tumors. The second case reported by Bergstrand offers the additional observation that there was a diminished density of the bones of the foot in which the multiple tumors occurred, which resembled multiple cysts. The tumors in his case occupied an intra-osseous position. This occurrence had not been reported previously, though others had observed rarefaction of the phalanges, as observed by Roentgen rays, at the site of the tumors. The pressure exerted by the expansile tumor is probably the cause of the demineralization of the contiguous bone. This appears to be a form of pressure atrophy (Grauer and Burt) rather than an atrophy of disuse, as was suggested by Stabins.

Grauer and Burt review the physiologic properties of glomus tumors. Systematic attempts to determine whether vasomotor reactions are associated with glomus tumors were made by Stabins and his associates.²⁶⁸ They made local temperature studies before and after operation on 2 female patients who had subungual glomus tumors of the hand. Normally there is a synchronous drop in surface temperature in the hands on being immersed in cold water. However, after patients immersed their hands in water at 15° C., it was found that recovery to their previous temperature was more rapid on the involved side than on the uninvolved side; also that during immersion the temperature on the affected side did not drop as low as on the unaffected side. There was a difference of between 6° and 8° C. on the 2 sides. After operation, the temperature changes in the 2 hands were found to be synchronous. There was a vasodilatation in the skin of the affected member, which disappeared after the surgical removal of the tumor. However, the disappearance of the vasodilatation

did not occur until about 8 weeks after surgical excision. Owing to the persistence of some pain at the site of operation, associated with vasodilatation, the authors considered the vasodilatation of the tumors as being caused by the painful sensory stimuli. This belief was strengthened by the fact that the vasodilatation disappeared when the pain ceased. Mason and Weil cited a case of Paulian and his associates in which there was an increased feeling of warmth and perspiration over the whole right side of the body in which the increase in temperature of from 0.5° to 2° C. was observed on the affected side. These changes disappeared following operation. They studied a person aged 69 who complained of intermittent claudication as a result of peripheral vascular disease associated with senile arteriosclerosis. Following treatment of alternating positive and negative pressure the circulation improved and the thermocouple temperature became normal. Six weeks after treatment for the claudication, a glomus tumor appeared on the leg. This was associated with sharp, agonizing pain and was followed by a peripheral drop in temperature, probably owing to an arterial spasm caused by the tumor. Excision of the tumor brought prompt relief and a return of the peripheral temperature to normal. The observations emphasize the physiologic effects produced by these tumors and indicate the association of the glomus with the maintenance of the temperature of the skin, whether through dilatation or constriction of the vessels. They suggested that under pathologic conditions congestion causes swelling with pressure on the nerves and consequent paroxysms of pain, while cold causes contraction of the muscular walls of the vessels with compression of the nerve fibers between the glomus cells and resulting pain. In either event, pressure is the important precipitating factor and whether or not the disturbance is a central one with involvement of the sympathetic system is still a moot question.

An excellent discussion of the pathology of the glomus is given by Weidman and Wise. The study of this article cannot be urged too strongly upon the reader.

"In brief, the neuromyo-arterial glomus is a normal vascular anastomosis which is distinctive in two respects: (1) it is arterio-venous, *i. e.*, there are not any intervening capillaries, the structure having a special and peculiar architecture; and (2) it includes special arrangement of muscle and nerve tissue. It occurs almost exclusively, as known at present, on the extremities, both upper and lower. It is supposed to play an important part in heat regulation. It assumes dermatologic significance when it becomes neoplastic, under which circumstances it is the vascular phase of this neuromyo-arterial complex which leads to the objective symptoms. It is probably the nerve phase of the complex which is responsible for the intolerable pain."

The glomus tumor, according to Weidman and Wise, is thus merely an exaggerated and disorderly glomus, attended by hyperplasia of its elements. It is exaggerated in that in most glomus tumors there is a new formation of Suquet-Hoyer canals. In a single histologic section there may be hundreds of larger and smaller ones, variously deformed and more or less intercommunicating, each being surrounded by its mantle of epithelioid cells and nerve fibers. It is disorderly in that the Suquet-Hoyer canal is not separately and purposefully aligned with the afferent artery on the one hand and with the primary collecting vein on the other. It is further disorderly in that the mantle of epithelioid cells is not at all uniform, *i. e.*, is thin in some places and excessively thick in others.

Weidman and Wise agree with Mason and Weil and others that this tumor should be regarded as a hamartoma rather than as a true neoplasm. The question of malignant change in glomus tumor is still *sub judice*. Ostrowski believes that a critical attitude should be maintained on this point and although no cases of malignancy following these tumors has been observed, it is still a possibility

Love described a pin test for the diagnosis of glomus tumor:

"An ordinary steel pin is used. The patient is asked to indicate the maximal pain or tenderness. Oftentimes as the physician passes his finger toward the spot of the maximal pain, the patient will withdraw his extremity just as the patient with trigeminal neuralgia will withdraw his face when the trigger zones are approached. However, when the patient's confidence is gained and the point of a steel pin is used to examine around the lesion, the point of the pin can be pressed into the skin as near as 1 cm. to the lesion without producing severe pain, but just as soon as the point is pressed over the lesion, the patient will have an excruciating attack of the characteristic pain projecting from the lesion." One may be able to identify the lesion when it is not visible.

The glomus tumor can be removed only by complete surgical excision. Adair attempted to apply radiation in 1 case, using 1 square radium plaque of 700 mc. hours at 1 cm. distance and a 3 mm. brass filter. This treatment was repeated but caused no change in the tumor. Lewis and Geschickter applied 4 courses of Roentgen rays to a glomus tumor which had previously been diagnosed as Ewing's tumor, with no satisfactory results. One of the cases of Stabins and his co-workers failed to respond to 50 mg. hours of radium unfiltered. Stabins considered this as a therapeutic test verifying the diagnosis of glomus tumor. In 1 of Graves' and Burt's cases, which was at first considered hem-angio-endothelioma, 4 courses of Roentgen treatment were given without any change in the size or character of the tumor. Two months after this treatment, the true nature of the tumor occurred to them. The stubborn resistance of the glomus tumor to all means of irradiation would tend to strengthen the objection to the so-called epithelioid cells being cells of embryonic character, particularly angioblasts, since all such cells are radiosensitive. Differential stains did not enlighten the authors as to the possible origin or nature of the glomus cells.

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OTO-RHINO-LARYNGOLOGY

UNDER THE CHARGE OF

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THE DECLINE OF ACUTE SURGICAL MASTOIDITIS

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AN analysis of the more recent communications appearing in the otologic literature indicates a decline in the incidence of acute surgical mastoiditis. Directly responsible for the decline is the widespread utilization of sulfonamides and penicillin—this despite such factors as seasonal variations, differing degrees of bacterial virulence, inadequate dosage, masking of symptoms, drug resistance, and toxic reactions. Indeed, the tenor of the current state of affairs may be summarized in the words of Bowers,^{4a} who during the course of a presidential address before the American Otological Society declared: "With the advent of chemotherapy, the number of cases of mastoiditis and its sequelæ—particularly meningitis—has steadily declined. . . . The great decline in cases has already made it difficult to teach our resident physicians how to perform adequate mastoidectomies."

Despite the understandable conservatism of most otologists, pediatricians in general felt that sulfanilamide prevented extension of infection from the middle ear to the mastoid cells when treatment with sulfanilamide was employed during the winter of 1937-1938. In 1938 Horan and French⁶ wrote of their experiences in an English naval hospital: the incidence of mastoiditis had fallen from 22.7 to 4.5% following the use of sulfanilamide in the treatment of acute purulent otitis media. The following year Fisher⁷ presented a report on 180 cases: 92 patients were treated in the ordinary

manner, and 88 were given sulfanilamide in addition to the usual form of treatment. In the group without sulfanilamide therapy there were 66 mastoidectomies. Mastoiditis developed in only 7 of the 88 patients who were given sulfanilamide.

In 1940, in a discussion of the rôle of chemotherapy in the treatment of complications of acute middle ear suppuration, Lindsay¹⁰ stated: "The experience at the University of Chicago Clinics during the past 3 years has been that the number of operations necessary for acute mastoid suppuration has dropped materially, which apparently corresponds with general experience throughout the country." In 1942 De Sanctis⁵ and his associates made a survey of 1992 cases of otitis media and mastoiditis in children hospitalized from 1931 to 1941. Comparison of the groups treated with and without sulfonamides from 1937 to 1941 showed a 9% incidence of mastoiditis in the former and 30% in the latter. Thus sulfonamides have played a dominant rôle in these changes of incidence. Bilchick and O'Kane² described a study in which 40 patients were treated with sulfanilamide, with 40 controls. The duration of discharge was reduced by one-third in the treated group, and the number who underwent mastoidectomy was reduced by one-half. Bowers^{4b} employed chemotherapy over a 3 year period in 50% of his 793 cases of acute otitis media. The incidence of mastoidectomy was reduced about 50%. Boies³ reviewed his experiences with

650 cases of acute surgical mastoiditis during the years 1931 to 1941. From a study of the literature he concluded that a majority of the otologists and a larger majority of the pediatricians believe that sulfonamide therapy has been responsible for a distinct decrease in the incidence of acute otitis media and in the necessity for mastoid surgery in the mastoiditis complicating otitis media. Boies sent out a brief questionnaire to 30 otologists whose writings, or association with a public ward or teaching service, or geographic location made their viewpoints of special value. To the question "How much of a decreased incidence of acute surgical mastoiditis have you experienced in the past year or two (1940-1941)?" the answers were unanimous in the matter of decrease, with estimates of from 25 to 95%, particularly in private practice.

An investigation described by Falbe-Hansen and Becker-Christensen⁶ in 1944 covered a total of 658 cases of acute suppurative otitis media, 323 in the control group and 335 in the treated group. The total number of patients included 419 under 15 years of age; 194 patients were treated with sulfapyridine, 31 with sulfathiazole. The average period of treatment was 7 days. The 3 organisms most frequently encountered in the pus from these ears were pneumococci, hemolytic streptococci, and Pfeiffer's bacilli. In the group treated with sulfapyridine, the period of discharge was reduced by 28%. Excluding patients who vomited, the reduction of discharge period was 40%. In the group treated with sulfathiazole, where vomiting was a rare occurrence, the reduction in discharge period was likewise 40%. In the control cases 9.3% required mastoid surgery, whereas in the groups treated with sulfonamides, 1.5% required operation.

A current review by House⁹ of 3326 case histories of patients hospitalized at the Los Angeles County General Hospital because of otitis media before and after the general therapeutic use of sulfonamide drugs began elicited the following informa-

tion: The number of patients hospitalized because of otitis media has decreased by 50%. In 4 of 5 patients with acute otitis media the pathologic process can be arrested within 3 days if the administration of sulfonamide compounds is instituted at the onset of symptoms. The incidence of mastoidectomy in patients hospitalized because of acute otitis media has been reduced by two-thirds. Spontaneous recovery occurs 1 week earlier in patients with acute otitis media who are treated with sulfonamide compounds. Surgical intervention was necessary approximately 1 week earlier when sulfonamide compounds were not administered. Postoperative drainage has been lessened by 1 week. The incidence of complications in cases of acute otitis media has been reduced by 20%. The mortality rate of otitic complications has been reduced by one-half. Before the advent of sulfonamide compounds as therapeutic agents, 9 of 10 persons with otitic meningitis died. With the therapeutic employment of sulfonamide compounds the rate is 1.5 to 10.

According to Weinstein and Atherton,¹⁴ acute suppurative otitis media is a problem of great importance to physicians who encounter it either as an isolated disease or during the course of such infections as scarlet fever or measles. Improperly or inadequately treated, its complications may lead to partial or total loss of hearing and in some instances may seriously endanger life. Treating 50 cases of acute suppurative otitis media with penicillin, they found that complications were reduced to a minimum. Mastoidectomy was only an infrequent episode. In the experience of Swanson and Baker,¹³ the use of penicillin frequently made it possible to avoid surgical intervention for acute mastoiditis. Smith¹¹ asserted that in cases of acute otitis media with clinical and roentgen evidence of mastoiditis, the clinical response to penicillin was prompt and often dramatic. It was believed that the drug was an important factor in cur-

ing the infection and sparing the patient mastoidectomy.

If penicillin is given early in acute otitis media, Struble¹² finds that the infection may be aborted. It must be given in adequate doses and over a sufficiently long period to prevent development of penicillin-resistant strains of bacteria and relapses. If given late, it may mask the infection and symptoms. If the surgical indications for drainage are still present, one resorts to myringotomy and mastoidectomy.

Twelve patients with acute suppurative otitis media complicating scarlet fever were treated with a sulfonamide compound by Ball¹; penicillin was administered only after the infection had been demonstrated to be resistant to sulfonamide drugs. Of the 12 cases of acute otitis media complicating scarlet fever, 1 required surgical intervention. In 10 of the cases the otitis media responded rapidly to treatment; in 1 case recovery

took place slowly. The 10 cases in which response to treatment was rapid showed resolution of the involvement of the tympanic membrane and restoration of normal hearing in from 1 to 2 weeks (average time 9 days). Ball considers the prompt resolution and healing of acute non-scarlatinal suppurative otitis media complicated by mastoiditis, without the necessity of surgical intervention, most promising. Five cases of acute non-scarlatinal otitis media complicated by mastoiditis were studied. Each patient had been under treatment with a sulfonamide compound and was given penicillin only after signs and symptoms of complicating acute mastoiditis had developed. Cessation of aural suppuration, abatement of all subjective signs and symptoms, and complete resolution of the process in the tympanic membrane were accomplished in from 3 to 18 days without resorting to surgical intervention.

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BOOK REVIEWS AND NOTICES

A TEXTBOOK OF BACTERIOLOGY AND IMMUNOLOGY. By JOSEPH M. DOUGHERTY, Ph.D., Professor of Bacteriology, Villanova Coll., and ANTHONY J. LAMBERTI, Instructor in Bacteriology, Temple Univ., School of Medicine. Pp. 360; 102 illus. St. Louis: Mosby, 1946. Price, \$4.50.

DESIGNED for pre-medical students or nurses studying medical bacteriology, this textbook offers the usual textbook material including a chapter on parasitic protozoa, on water, milk, and food examination, but nothing on pathogenic fungi. The content of the book is altogether too brief for undergraduate medical bacteriology. It stresses the early historical events in bacteriology and omits too many important modern developments and details. The greater part of the text could have been written more than 6 years ago without change of its present form. Exceptions are the chapters on chemotherapy, tuberculosis, and protozoa. The chapter on viruses considers only 4 virus diseases and bacteriophage. The rickettsioses considered are only epidemic typhus and Rocky Mountain spotted fever. To illustrate immunity, the old diagrams of Ehrlich are presented without consideration of recent advances. Inaccurate and misleading statements abound. Thus the statement is made that "typhus fever never develops in the absence of human body lice," and subsequent treatment omits murine typhus completely. Judging this book in its entirety, it cannot be recommended for student use. J. F.

ORAL MEDICINE. By LESTER W. BURKET, M.D., D.D.S., Professor of Oral Medicine, The Thomas W. Evans Museum and Dental Institute, School of Dentistry, University of Pennsylvania. Pp. 674; 350 illus. Philadelphia: Lippincott, 1946. Price, \$12.00.

THIS is an excellent presentation of the various oral diseases and the various oral aspects of internal medicine. The book is well organized, comprehensive and thoroughly practical, as outlined in an introduc-

tion by J. L. T. Appleton. The first 200 pages deal with the various diseases of the oral mucosa, such as gingivitis, fusospirochetel infection, and the oral manifestations of metal poisoning, allergy and various skin diseases. The following 100 pages are devoted to the various diseases of the respiratory, cardiovascular, gastro-intestinal and urogenital systems. Then follow the disturbances of the endocrine glands and the diseases of the bones and joints. The remainder of the text is concerned with the vitamin deficiencies, the blood dyscrasias, the specific infectious granulomata, focal infection, oral pediatrics and gerodontics. The book is richly illustrated. As a special feature it is furnished with a color atlas showing a selected group of 60 commonly encountered oral lesions. Another feature is a regional diagnostic index of the more common sites of the various oral manifestations. Everywhere oral changes are presented as part of systemic disease. The book is highly recommended to dentists and physicians alike. It is felt that it is a "must" for all practitioners of the healing arts.

W. E.

HUMAN EMBRYOLOGY (PRENATAL DEVELOPMENT OF FORM AND FUNCTION). By W. J. HAMILTON, M.D., D.Sc., F.R.S.E., Professor of Anatomy, Medical College of St. Bartholomew's Hospital, University of London; J. D. BOYD, M.A., M.Sc., M.D., Professor of Anatomy, Medical College of the London Hospital, University of London; and H. W. MOSSMAN, M.S., Ph.D., Associate Professor of Anatomy, University of Wisconsin. Pp. 374; 364 ills. Baltimore, Williams & Wilkins, 1945. Price \$7.00.

THIS is a welcome addition to the small group of current texts on human prenatal development. It is a wartime product of coöperation between British and American scholars and is a credit to all concerned. The authors show themselves to be thorough anatomists, interested in physiology, clear in their descriptions and modern in their

viewpoint. The morphology is unusually well illustrated by the English medical artist, A. K. Maxwell, who is very successful in his three dimensional pictures. These are important for the student, who has little opportunity of studying human embryology in the laboratory and for whom in consequence the descriptive text may be insufficient. For those interested in following up special topics the opportunity is provided by over 500 references to journals and books, given at the ends of chapters. Of the 16 chapters in the book the first 3 are concerned with germ cells and the cyclic changes in the female genital tract. Chapters 4 to 7 give the early stages of human development in the light of contemporary studies and include a photograph of the $7\frac{1}{2}$ day human ovum of Rook and Hertig. The illustrations are from modern sources and replace the classic figures which have adorned text books of embryology for the last half century. Among these sources the publications of the Carnegie Laboratory of Embryology are prominent. Chapter 8 is concerned with theories and experiments since the time of Roux and bears the title, "Determination, Differentiation, The Organizer Mechanism, Abnormal Development and Twinning." These represent the modern attempt to learn something of the forces that underlie the complex changes which result in the functioning individual. Chapters 9 to 15 give the organogeny of the several systems, and here again one is impressed with the clearness of the illustrations and the effective use of color. The last chapter is entitled Comparative Vertebrate Development and shows useful figures of the fetal membranes and placenta in domestic animals such as pig, sheep, dog, rabbit and other rodents. This book is designed for the serious student, but all can profit from it and especially from the illustrations.

W. A.

AN OUTLINE OF ORGANIC NITROGEN COMPOUNDS. By ED. F. DEGERING and Collaborators, Department of Chemistry, Purdue University. Pp. vi, 752. Ypsilanti, Mich.: Lithoprinters, 1945. Price, \$7.50.

THIS book which presents in outline form the chemistry of the important nitrogen compounds is more than a revision of the volume which appeared under the same title in 1942. It is essentially a new book with

respect to content and organization. There are 45 chapters dealing with such topics as General Concepts, The Fixation of Nitrogen, The Ammonia System of Compounds, The Nitroalkanes, Polynitroalkanes, Aromatic Nitro Compounds, Oximes, Aliphatic Amines, Amino Acids, Polypeptides and Proteins, the Diazenes, Aromatic Amines, Alkanolamines, Aromatic Diazo and Diazonium Compounds, Hydrazines, Urea and Its Derivatives, Guanidines, Organic Nitrogen Dyes, Alkaloids, Vitamins Containing Nitrogen, Synthesis of Nitrogen Ring Compounds, as well as many other related categories.

Each chapter is subdivided into sections on: (1) history, occurrence, structure, uses; (2) nomenclature; (3) methods of preparation; (4) physical properties; (5) reactions.

This outline will undoubtedly be more useful as a reference than as a textbook. It contains a wealth of material systematically arranged for the research chemist. S. G.

THE PHYSIOLOGICAL BASIS OF MEDICAL PRACTICE. By CHARLES H. BEST, C.B.E., M.A., D.Sc. (London), F.R.S., F.R.C.P. (Canada), Professor and Head of Department of Physiology, Director of Banting-Best Department of Medical Research, University of Toronto; and NORMAN BURKE TAYLOR, V.D., M.D., F.R.S. (Canada), F.R.C.S. (Edin.), F.R.C. (Canada), M.R.C.S. (England), L.R.C.P. (London), Professor of Physiology, University of Toronto, Canada. Pp. 1169. Fourth ed. Baltimore: Williams & Wilkins, 1945. Price, \$10.00.

THE world wide recognition that this lusty 9 year old has acquired hardly needs the confirmation of the "four editions and fourteen printings since 1937" and of Spanish and Portuguese editions. The linking of laboratory and clinic, very much in the limelight in these curriculum conscious days, is undoubtedly a large factor in the book's desired popularity. The emphasis on morbid physiology, rather than morbid anatomy is another indication—present in all editions to be sure—of the author's appreciation of modern trends and values. Up to date anatomists, physiologists, pathologists and internists should all feel at home in this wide-embracing volume. Though with its present additions it has now almost reached

maximum size, its small type (made tolerable by the use of double columns and generous heading) allows inclusion of an amount of material seldom found in a single volume.

The author's terse graphic style, the well chosen space-saving illustrations and the 57 pages of references, all add to the attractiveness of this excellent work. E. K.

SCIENTIFIC, MEDICAL, AND TECHNICAL BOOKS PUBLISHED IN THE UNITED STATES OF AMERICA, 1930-1944: A SELECTED LIST OF TITLES IN PRINT, WITH ANNOTATIONS. Edited by R. R. HAWKINS. Prepared under the direction of the National Research Council's Committee on Bibliography of American Scientific and Technical Books. Pp. 1114. Washington, 1946. ("Any inquiries concerning this bibliography may be addressed to United States International Book Association, 27 East 67th Street, New York City 21.")

CONCEIVED in the midst of war, this annotated list of approximately 6000 books (an equal number of available titles was rejected for one reason or another) has been brought to term in the amazing period of less than 2 years—approximately a year later than its sponsors had hoped, at that. In no other country in the world, probably, would a bibliographic project of such scope have been even attempted under like conditions—still less, achieved. Our American publishers, who succeeded in enlisting the aid of the Department of State, the National Research Council, and the Office of the Coördinator of Inter-American Affairs in the project, have reason indeed to congratulate themselves.

The list is intended primarily to provide other countries with descriptions of important scientific books published in the United States in the given period—that are still in print. The latter qualification, the omission of publications issued by states and territories, and the exclusion of some branches of the subjects treated, necessarily prevent the list from presenting a completely rounded and truly definitive picture of American scientific publications in these years. It is obvious, on the other hand, that a publication of this sort would not have been feasible at all, had not some restrictions been enforced. The list should admirably satisfy its primary purpose; but it is to be hoped that its clearly stated limitations will

not be overlooked, now or subsequently, by researchers, librarians, and historians, to all of whom it can be very useful without serving as a definitive bibliography.

The annotations, which follow rather full analyses of the contents, "came," as the editor says, "from a variety of sources and inevitably reflect their origins." Many recall the language of the publishers' catalogues and announcements; some contain a fleeting critical comment (*e. g.*, "the style is dignified and sober"), but probably most are as objective as the purpose warrants. There are author and subject indexes, and an Appendix containing a directory of state agencies and a directory of publishers. The typography of the weighty volume has been excellently designed by Carl P. Rollins, Printer to Yale University. Of the editor and of the successive chairmen of the National Research Council's Committee (Harrison Craver, Hon. Sc.D., and John F. Fulton, M.D.), one can only say that the volume is an impressive tribute to their respective skills and stamina.

Having made such a handsome beginning, cannot all the forces concerned coöperate to keep the list up-to-date by issuing, say, quarterly or semi-annual supplements, which need not now suffer most of the restrictions enforced by the times on the present publication? If that were done, we should be offering to the world of science an even more valuable tool in the form of a reasonably definitive bibliography unique in our time and useful for all time. That would be wise and very practical diplomacy indeed.

W. McD.

THE ELECTRON MICROSCOPE. An Introduction to Its Fundamental Principles and Applications. By E. F. BURTON, Univ. of Toronto; and W. H. KOHL, Rogers Electronic Tubes, Ltd. 2nd ed. Pp. 325; 125 drawings, 136 light and electron micrographs. New York: Reinhold, 1946. Price, \$4.00.

THIS book is written by the builders of the first electron microscope in America. It presents the elements of electron microscopy to the layman, advanced high-school students and members of those professions which have been influenced by this new science. The nature of light and of electron beams and the methods of focusing them are dealt with in a non-technical fashion,

the text being amply illustrated with cartoons and line drawings. The excellent selection of electromicrographs, biologic, bacteriologic, chemical, and metallurgic specimens is printed on high quality paper and represent a marked improvement over the 1st edition. While the significance of the micrographs is touched upon very lightly, an Appendix contains a complete bibliography of the entire field of electron microscopy up to 1944 for those who wish to delve further into particular developments in the field.

T. A.

NURSING AND NURSING EDUCATION. By AGNES GELINAS, R.N. Pp. 72. Philadelphia: Commonwealth Fund, 1946. Price, \$1.00.

THIS monograph marks an era in nursing which might be called its "coming of age." It is noteworthy that it originated in The Committee of the New York Academy of

Medicine on "Medicine and the Changing Order." The inclusion of nursing both emphasizes its inseparable relationship to medicine and recognizes the emergence of nursing as a separate profession.

The contacts deal with the development of nursing; supply and demand in both world wars; personnel policies and recommendations including those for auxiliary workers; estimated supply and demand, and the future building of nursing.

This book is recommended for thoughtful study by nurses, physicians, and all concerned with the health of the community. It should be particularly helpful to members of Boards of hospitals and public health agencies.

The selection of the author as spokesman for nursing is a tribute to her proven ability in nursing education as well as her personal acceptability to both medical and nursing groups.

T. L.

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ORIGINAL ARTICLES

COMPLICATIONS FOLLOWING THE ADMINISTRATION OF THIOURACIL*

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AND

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REPORTS of unfavorable complications occurring during the administration of thiouracil have appeared in the literature ever since the first clinical trial of this new drug in patients with toxic goiter by Astwood.¹ It has become increasingly apparent that the most important untoward reaction to thiouracil is the development of agranulocytosis, which is always a serious complication and one which may terminate fatally; all others are of minor importance in comparison to it. Since the incidence and mortality rate of this condition will undoubtedly be a significant determining factor in the future use of the drug, it is our purpose to devote the main part of this report to a consideration of the reasons for a fatal outcome in a few patients with this disease, whereas a majority who have developed this disorder have survived. In addition, other less serious reactions will be reviewed in the light of our experience with 80 patients treated with the preparation. From the literature and through 2 personal communications 9 fatalities occurring during or following thiouracil administration have been collected.^{3,5,7,8,10,12,17,18}

Inspection of the data in Table 1 shows

that 7 of the 9 fatalities were definitely attributable to agranulocytosis. The first case of Himsworth's should be excluded because there was a leukocytosis, neutrophilia and pneumonia. Also the case mentioned by Sevringhaus did not succumb from agranulocytosis but died in thyroid crisis following an episode of the disorder.

In an attempt to determine if all of the above 7 fatal cases had 1 or more significant factors in common which could account for the fatal outcome of the condition, many possible underlying causes were given careful consideration. Apparently the age which varied from 41 to 72 years, the sex, and the presence of various complicating diseases did not play an important rôle. In the case reported by Stock *et al.*,⁸ however, it was admitted that the patient had severe diabetes, which might have been an important contributing element in the death of the patient. During the first 2 years in which thiouracil was employed, it appeared as though excessive dosage might be a major cause of the fatalities.^{1,3,12} This impression, however, is probably incorrect as more recently deaths have resulted from the administration of doses as small as 0.2 gm. daily.^{5,7}

* Part of the expense of this study was defrayed by a grant from the Upjohn Company, Kalamazoo, Michigan.

TABLE 1.—ANALYSIS OF 9 FATALITIES OCCURRING FOLLOWING THE ADMINISTRATION OF THIOURACIL
(The cases are listed by authors as collected from the literature and through personal communications)

Age Sex Complicating conditions	Author	Kahn and Stock 62 years 1 female Hypertension, diabetes (mad con- trol) +0.65% 0.1 gm./dl.	Lozinski and Siminovich 49 years 1 female Auric fibrillation, ? coronary thrombosis +30% 0.9 gm./dl. (after Lugol's) Weak, depressed, chill fever, sore throat, temp 102.2°	Ferrer, Span and Cathcart 70 years Male Emaciated, cardiac enlargement with auric fibrillation +40% W B C 1250, 32% P, adm. in 2 days temp up to 105°; sore throat	Lahey and Bartels 41 years 1 female 0.6 gm Sore throat 1000 0% ? days (poss same) 5 days Penicillin last 8 hrs. of life Penicillin given too late
Initial B M R Doseage at time of reaction Symptoms of toxicity at start of reaction		1100 2% (N), 1% (T) 48 hours 8 days	900 0% 2-3 days 10 days	460 0% ?	?
Lowest W B C and neutrophil count Time from onset of symptoms to drug cessation Time from onset of symptoms to death				5 days (7 days from onset of decreased W B C) Whole blood, penicillinoloid; penicillin (last 24 hrs)	
Treatment		Whole blood, crude liver, penicillinoloid, yellow bone marrow; penicillin (when ?) "Severe diabetes mellitus is believed to have been a contributing factor in the death of this patient"	Whole blood, liver extract, penicillinoloid Terminal pneumonia, no penicillin	Penicillin much too late, transfusion reaction with hb, nephrosis found at postm.	
Comment					

Age Sex Complicating conditions	Author	Hinsworth 43 years 1 female +26.24 0.6 gm./dl. Malaria, temp to 104°, chills 1500 0% 3 days 9 days	Hinsworth ? female Cardiac enlargement +24 0.2 gm./dl. "Pneumonia" 0% 5 days 7 days	Gargill and Lesses 56 years 1 female Thiouracil, induced myx- edema 30% 0.2 (symp on 3rd course) Sore mouth and throat, shaking, chill, temp 103.5° 900 0% 2 days 8 days	Tiller 72 years 1 female B P. 190/90 55% 0.6 gm 2000 0% 24 hours 10 days (from abn et.)
Initial B M R Doseage at time of reaction Symptoms of toxicity at start of reaction					
Lowest W B C and neutrophil count Time from onset of symptoms to drug cessation Time from onset of symptoms to death					
Treatment		Blood transfusions, penicillinoloid, sulfadiazine last 24 hours Death from pneumonia with W B C 25,000, neutrophils 75%	Sulfathiazole (X 7 da ?) Death pneumonia and agnucloeo- tosis	Sulfadiazine, sulf ismerazine, blood transfusions, penicillin (on 3rd day) Penicillin too late	Transfusions, penicillin 24 to 18 hrs after onset of symp, crude liver, penicillinoloid Syp on adm temp 104° and abd pain, no sore throat, jaundice soon developed, no post- mortem
Comment					

It is our present impression, therefore, that while excessive dosage must be kept in mind as the cause of fatal agranulocytosis, it is not the sole determining factor. Recently Limarzi¹¹ reported his observations on 1 case of myelogenous leukemia who developed a severe leukopenia following the administration of 3 gm. daily for 3 months. He concludes that thiouracil in sufficient doses regularly inhibits granulocytosis and destroys granulocytes, which is interpreted as an effect independent of individual susceptibility. Although this statement may prove to be correct in the light of further experience, such a conclusion does not seem justifiable at present.

A review of the 7 untoward fatal cases of agranulocytosis associated with the administration of thiouracil shows that there are at least 5 important contributing factors to the fatal outcome. These are tabulated in the following paragraphs:

1. FAILURE TO ADMINISTER PENICILLIN PROMPTLY. In not 1 instance was treatment with penicillin given from the onset of symptoms to death, nor even following the discontinuance of thiouracil to the time the patient expired. In all cases in which this form of therapy was administered, it was given *as a last resort*. The great significance of this factor is obvious from the knowledge that the average length of life after onset of the symptoms of a thiouracil reaction was 8 days, with the shortest interval being 5 days and the longest one 16 days. The opportunity for instituting therapy with this most effective agent was, therefore, to a certain extent, regrettably overlooked in each patient.

2. DISCONTINUANCE OF THE DRUG AFTER THE ONSET OF SYMPTOMS. Since fully developed agranulocytosis may be present within 3 days after the onset of the initial warning symptoms, it is apparent that the drug should be discontinued, temporarily at least, with the appearance of the very earliest evidence of an untoward effect. In all of the tabulated fatalities, with the possible exception of the case of Lahey

and Bartels,¹⁰ the drug was discontinued after an interval of 24 hours to 5 days following the recorded onset of symptoms, or an average of at least 2 days. It is possible that all these deaths might have been averted if the drug had been stopped promptly after the appearance of the ominous symptoms.

3. DISREGARD OF SIGNIFICANT SYMPTOMS OF IMPENDING AGRANULOCYTOSIS. Six of the 7 patients experienced definite symptoms which should have served definitely as an indication to the intelligent and forewarned patient to discontinue the drug promptly and seek medical advice. In the seventh patient the information is incomplete and hence it is not possible to determine from the record if such manifestations were present. It should be emphasized that any symptom, regardless of its nature, when it appears in a patient who is receiving thiouracil, should be regarded tentatively as due to the action of the drug and hence should be investigated thoroughly. This means, in addition to other things, that a white blood cell count and the differential formula of the leukocytes should be determined. From a study of the fatal cases, it was found that fever, sore throat, and malaise were the 3 most common manifestations of this disorder, named in order of frequency.

4. INEFFECTICACY OF VARIOUS FORMS OF THERAPY FOR AGRANULOCYTOSIS. One or more of the recommended forms of treatment of agranulocytosis, namely, the sulfonamides, crude liver extract, pentnucleotid, whole blood, yellow bone marrow, and folic acid was used in each case without preventing death. This and our successful use of penicillin in our 3 cases of agranulocytosis suggests that penicillin is undoubtedly the most effective type of therapy that can be used in combating the sepsis of agranulocytosis which is the usual cause of death in the disease.

5. FAILURE TO DISCONTINUE THIOURACIL WHEN A SEVERE LEUKOPENIA DEVELOPS. Although the majority of patients with thyrotoxicosis have a mild to moderate leukopenia and lymphocytosis, it is

evident from our experience that when the total white cell count drops below 2000 per c.mm. during thiouracil therapy, it is an indication that the patient is in grave danger. It is especially important to have a white blood cell count and differential blood count done before thiouracil is instituted in order to evaluate more accurately the subsequent course of events.

In a survey of the 7 fatalities, it has been found that 5 common facts, given above, if kept in mind by the physician, should enable him to avert fatal reactions or reduce them to a minimum. It is striking to note that each fatality occurred in patients who were being treated by physicians with relatively little experience in the use of the drug. The 152 patients of Williams and Clute,¹⁹ and in our series of 80 patients, there were no fatalities although cases of agranulocytosis did occur. The 1 fatal case in the series of 190 patients of Lahey and Bartels¹⁰ occurred when the patient was no longer under their observation and in another hospital.

Non-fatal Hematologic Reactions. Of our 80 patients, 70 were treated with thiouracil as in-patients until the basal metabolic rate had fallen significantly and the clinical picture had improved considerably. These patients were hospitalized for a minimum period of 2 weeks and a majority for a much longer interval. A technician was employed to do total white blood cell counts and differential blood counts on these patients every other day. The largest daily dose of thiouracil given was 0.6 gm. After reviewing the literature, we feel that such a close check on the patient's clinical picture and blood status on such a large series has not been previously reported. It is our opinion that a careful review of the non-fatal hematologic reactions among these patients is worthwhile.

The only non-fatal hematologic reactions were those of agranulocytosis or neutropenic leukopenia. No decrease in number of the other circulating cellular elements of the blood occurred. One patient did

have thrombocytopenic purpura occurring *before* thiouracil was started. This disappeared during thiouracil therapy.

These observed non-fatal hematologic reactions occurred 6 times in 5 patients, or in approximately 8% of the group. This is a definitely higher incidence than in 3 of 152 (2%) in the series of Williams and Clute¹⁹ and in 9 of 190 (4.7%) in the series of Lahey and Bartels;¹⁰ the patients in both of these series were followed for the most part as out-patients. We attribute this difference in incidence to the fact, reported by others,^{2,13} that mild non-symptomatic neutropenia and neutropenic leukopenia may occur occasionally in some patients and disappear spontaneously despite the fact that the thiouracil medication is not discontinued.

Neutropenic Leukopenia (Tables 2, 3 and 4). This hematologic reaction occurred as frequently in our series (3 cases) as did true agranulocytosis, which was observed 3 times in 2 patients. Here, as in the fatal cases of agranulocytosis, the hematologic reaction was always preceded or accompanied in its earliest phase by a drug fever with or without skin eruption, sore mouth and other symptoms. Also, the full development of the symptoms occurred in the brief interval of 3 days. The importance of this fact was made obvious in the discussion of the fatal cases of agranulocytosis. This observation also illustrated that occasionally the simple act of withholding thiouracil for as short a time as 1, 4 and 8 days may be the only change necessary in the successful management of the patient. Our view in regard to this is in accord with the experience of Palmer¹⁵ who resumed the administration of the drug after 72 hours in each of her first 9 cases of neutropenia and leukopenia with satisfactory results.

Agranulocytosis (Tables 5, 6 and 7). Our charts show 3 episodes of agranulocytosis which developed in 2 patients. The second reaction in the first patient is the sole example observed in our group of a second attack of agranulocytosis in the same patient, resulting from thiouracil.

We think, however, that a recurrence is an important danger to keep in mind since it accounted for 1 out of the 6 reactions. In these 3 episodes of agranulocytosis, the warning symptoms were again present in all cases. The agranulocytosis in each instance developed within 3 to 5 days

after the appearance of the premonitory symptoms, and recovery occurred in 6 to 7 days following the use of penicillin alone. This group and the preceding one raise the question concerning the value of certain therapeutic measures, such as liver and folic acid, as suggested by Goldsmith

TABLE 2.—NEUTROPENIA OCCURRING DURING THIOURACIL ADMINISTRATION WITH SYMPTOMS OF AGRANULOCYTIC ANGINA, DERMATITIS, AND DRUG FEVER

A. R. No. 463451.

Date	Dosage per day (gm.)	Symptoms	Temp. (° F.)	W.B.C.	Neutrophils (%)	Therapy for reaction
4/14/44	0.6	99.6	8200	51	
4/15/44	0.6	Acneform dermatitis of face, pruritus	100.3			
4/16/44	0.6	Above inc. in extent and severity; sore throat	100.5			
4/17/44	0.2	Sore throat and dermatitis impr.	99.0	5000	37	Drug discontinued.
4/18/44	0.0	Pruritus persists	99.6	4800	53	
4/19/44	0.4	Still some pruritus	98.6	6650	69	

TABLE 3.—LEUKOPENIA AND NEUTROPENIA WITH DRUG FEVER ONLY

D. G. No. 549046.

Date	Dosage per day (gm.)	Symptoms	Temp. (° F.)	W.B.C.	Neutrophils (%)	Therapy for reaction
6/12/44	0.4	Temp. 99.3°-100° on admis.	99.3	6100	62	
6/15/44	0.4	99.4			
6/16/44	0.4	99.8			
6/17/44	0.4	100.0	4000	52	
6/19/44	0.4	100.7	3800	60	Drug deliberately continued
6/20/44	0.2	100.2	2250	55	
6/21/44	0.0	99.5	4150	45	Drug discontinued
6/22/44	0.0	99.0	3000	38	
6/23/44	0.0	99.0	3200	31	
6/24/44	0.0	99.0	4350	38	
6/25/44	0.2	99.0	4750	27	
6/26/44	0.4	99.0	5850	52	

TABLE 4.—LEUKOPENIA AND NEUTROPENIA WITH SYMPTOMS OF DRUG FEVER AND SORE THROAT

P. S. No. 550895.

Date	Dosage per day (gm.)	Symptoms	Temp. (° F.)	W.B.C.	Neutrophils (%)	Therapy for reaction
11/15/44	0.2	7000	49	
(Returns)						
11/28/44	0.0	"Hot all over"	101.0	2750	29	Drug discontinued
(Returns)						
11/29/44	0.0	Sore throat	Normal	1900	15	
12/ 1/44	0.0	None	Normal	3700	26	
12/ 2/44	0.0	None	Normal			
12/ 3/44	0.0	None	Normal			
12/ 4/44	0.0	None	Normal	4900	48	
12/ 5/44	0.0	None	Normal	5000	48	
12/ 6/44	0.0	None	Normal	5300		

TABLE 5. TOTAL AGRANULOCYTOSIS OF 5 DAYS DURATION WITH PREDOMINANT PICTURE OF SEPSIS

M. O. No. 550895.

Date	Dosage per day (gm.)	Symptoms	Temp. (° F.)	W.B.C.	Neutrophils (%)	Therapy for reaction
1/ 9 45	0.6	98.6	8550	76	
(Return)						
2/ 8 45	0.2	"Pain in neck;" ? No. of days; painful cerv. adenopathy; furuncle of nose, finger, forehead	102.5	3200	4	
(Returns)						
2/ 9 45	0.0	"Sore throat;" agranulocytic angina	103.4	1950	0	Penicillin 160,000 u./day
2 10 45	0.0	104.0	2100	0	Penicillin
2 11 45	0.0	105.6			Penicillin
2 12 45	0.0	103.5	1100	0	Penicillin and sulfadiazine
2 13 45	0.0	102.8	2950	0	Penicillin and sulfadiazine
2 14 45	0.0	103.0	2150	9	Penicillin and sulfadiazine
2 15 45	0.0	100.0	2450	40	Penicillin and sulfadiazine
2 16 45	0.4	98.0	4300	63	Penicillin only
2 17 45	0.4	99.0	5600	77	Penicillin only

*et al.*⁶ As a matter of interest, the third case of agranulocytosis (Table 7) occurred while the patient was receiving Armour's brewer's yeast (15 gm. t.i.d.). This material contains considerable folic acid and was administered purposely to prevent agranulocytosis.

It should be noted that the case in which thiouracil was used after the onset of

frequently in conjunction with other reactions. The reported incidence of attacks of fever in 4 series of patients from the literature is tabulated below.

Williams and Clute ¹⁸	2 in 152 (1.3%)
Gargill and Lesses ¹⁵	2 in 43 (4.6%)
Lahey and Bartels ¹⁰	7 in 190 (3.6%)
Paschkis ¹⁶	4 in 30 (13.3%)

TABLE 6.—TOTAL AGRANULOCYTOSIS OF AT LEAST 5 DAYS DURATION, ILLUSTRATING EFFECT OF NOT STOPPING THIOURACIL AT FIRST SYMPTOMS

M. O. No. 550895.	Dosage per day (gm.)	Symptoms	Temp. (° F.)	W.B.C.	Neutrophils (%)	Therapy for reaction
Date (Return)						
3/ 5/45	0.3	Routine count done by home physician; said to be "low;" thiouracil cont.				
3/ 8/45	0.0	Sore throat, temp. 103°; necrotic mouth ulcer; tender cerv. adenopathy	101.0	1280	0	
3/ 9/45	0.0	Sore tongue	102.4	1250	0	Penicillin 160,000 u./day
3/10/45	0.0	Sore tongue	103.8	1300	0	Penicillin 160,000 u./day
3/11/45	0.0	? slight icterus	103.5			Penicillin 160,000 u./day
3/12/45	0.0	103.5	900	0	Penicillin 160,000 u./day
3/13/45	0.0	104.0	1700	5	Penicillin 160,000 u./day
3/14/45	0.0	101.0			Penicillin 160,000 u./day
3/15/45	0.0	98.4	2450	29	Penicillin 160,000 u./day
3/16/45	0.0	98.6	4050	..	Penicillin 160,000 u./day

TABLE 7.—MARKED LEUKOPENIA AND NEUTROPENIA WITH PAROTITIS AND LOW GRADE FEVER AS WARNING SYMPTOMS

S. F. No. 566407.	Dosage per day (gm.)	Symptoms	Temp. (° F.)	W.B.C.	Neutrophils (%)	Therapy for reaction
Date						
6/15/45	0.6	99.2	5050	51	Brewer's yeast 15 gm., t.i.d. since starting thiouracil
6/16/45	0.6	99.2	4850	47	
6/17/45	0.6	99.0			
6/18/45	0.6	Parotitis, rt.	99.2	3750	22	
6/19/45	0.6	98.6	5150	32	
6/20/45	0.6	Angina, tonsil necrotic	99.0	3500	13	
6/22/45	Omitted	99.0	3350	11	
6/23/45	Omitted	100.0	2250	1	
6/24/45	Omitted	Rt. axillary abscess	102.8			Penicillin started
6/25/45	Omitted	Worse	102.4	2800	3	
6/26/45	Omitted	100.2	4600	7	
6/27/45	Omitted	99.3			
6/28/45	Omitted	101.6	7600	56	Roentgen ray to axilla

symptoms developed more complications (parotitis, fever, agranulocytic angina, axillary abscess) than the cases in which thiouracil was promptly discontinued.

Non-hematologic Reactions Which Required Cessation of Thiouracil and Thyroidectomy. Febrile Reactions. In only 1 of our 80 patients was it necessary to discontinue thiouracil because of febrile reaction. The febrile episodes which occurred in our patients developed between outpatient visits; hence they were not studied in detail, but from the history they resembled other reports in the literature. As noted above, fever was present more

The typical type of pyogenic reaction is portrayed by a case of Kenrick and Yater.⁹ A 17 year old colored female had a satisfactory response to thiouracil but experienced fever reaching 105° F. on 3 different occasions. The febrile attacks were accompanied by epigastric distress, low back discomfort, and pain in the eyes. The conjunctivæ were injected and there was profuse lachrimation. A thyroidectomy was then performed. Twelve hours post-operatively a dose of 0.2 gm. of thiouracil produced a typical febrile reaction, occurring 7 hours after the administration of the test dose. The authors conclude that

this was "probably a drug allergy" and not a typical toxic effect. The nature of this type of untoward effect needs further investigation before effective therapy can be recommended.

Cases Refractory to Thiouracil. No convincing reports of complete persistent resistance to thiouracil therapy have been published in the literature. In our group of 80 patients, we concluded that in 2 patients the results were only moderately successful and hence it was deemed advisable to perform a thyroidectomy. The case histories of these 2 patients are as follows:

CASE 1.—D. G., No. 549046, a 14 year old, white female, was admitted on June 6, 1944, with a history of shaking and nervousness since 1939 and with the onset of swelling in the neck in 1940. The goiter had progressed in size and in March 1944 the onset of blurring of vision was noted. On physical examination her skin was hot and moist. Exophthalmos was not present. The pulse was 126 per minute and the blood pressure 220/50. There was a fine tremor of the extended fingers and a diffusely enlarged thyroid gland. No definite evidence of cardiac disease was observed. The B.M.R. was +61 on June 7, 1944. Thiouracil was started in a dosage of 0.4 gm. daily on June 8, but 12 days later it was necessary to discontinue the drug for 4 days because of a cutaneous complication. At the end of this time, thiouracil therapy was resumed and the patient was kept in the hospital in bed until August 24, when her B.M.R. reached +16. She then continued this dosage for 2 weeks while resting at home, after which time it was reduced to 0.2 gm. daily. On December 6 the B.M.R. was +17. Her general condition had shown only moderate improvement and she had been attending school half-time. Thiouracil was continued in a dosage of 0.4 gm. daily and on April 13, 1945, her B.M.R. was -3%. Although her condition had improved greatly she was not entirely normal and hence thyroidectomy was performed.

CASE 2.—C. S., No. 568346, an 18 year old, white male, was admitted on May 9, 1945, with a history of onset of symptoms of thyrotoxicosis in November 1944. There was heat intolerance, insomnia, increased

appetite with weight loss of 30 pounds in 6 months, with nervousness and marked tremor. On physical examination his pulse rate was 130 per minute and blood pressure 155/80. The skin was warm and moist. There was no exophthalmos. Two nodules about 2 cm. in diameter were present in the thyroid. The B.M.R. on May 23, 1945, was +43, and after 3 days of bed rest was +50. Thiouracil was started on May 29 in a dosage of 0.6 gm. per day. He was discharged from the hospital receiving this amount on July 14. The B.M.R. on August 14 was +36 and on September 20 was +16%. The patient was readmitted on November 6, at which time the B.M.R. was found to be +9. The residual symptoms of thyrotoxicosis were present.

Both of these patients were young and had high basal metabolism rates with high pulse pressures. In both cases the B.M.R. was reduced to normal limits with thiouracil but not as rapidly as in the usual case. What is more important, the clinical condition did not improve sufficiently despite the prolonged periods of treatment. The first patient received 0.4 gm. daily or more during the 2 month period of hospitalization, 0.2 gm. per day for 4 months at home, and then 0.4 gm. per day for 4 months which is a total period of treatment of 10 months. In the second case the patient was maintained on maximum (0.6 gm.) doses of thiouracil for an interval of 5 months. He had a distinct tendency to improve during hospitalization but the symptoms became intensified on resumption of normal activities. The fact remains, however, that this patient could not be made entirely free of symptoms without an undesirably long period of hospitalization. Hence it was concluded that probably surgery offered the best opportunity for cure. Although we have found no mention of this fact in the literature, our experience gives the impression that the clinical response of out-patients is in general slower and less satisfactory than that of in-patients on thiouracil.

Carcinoma of the Thyroid Gland in Relation to Thiouracil Treatment. Carcinoma

has been reported to occur in from 2 to 11 % of patients with nodular goiters. Two of our 31 patients with nodular goiters were found to have adenocarcinoma of the thyroid at operation. In both instances the true diagnosis was suspected preoperatively. Nevertheless, although this represents an incidence of only 2.5 % of our total of 80 patients with toxic disease of the nodular and non-nodular types of goiters, it is an incidence of 6.6 % in patients with nodular goiter. For this reason it is our policy to employ thiouracil in patients with nodular goiters only as a preoperative measure. This procedure is followed in most large thiouracil series.

The Unreliable Patient. An ever-present reason for failure of thiouracil therapy is that a small percentage of patients cannot be relied upon to follow directions carefully. It is unsatisfactory to administer thiouracil to patients who are incapable of satisfactory coöperation, for at least 2 reasons. In the first place, such patients are likely to discontinue therapy or be irregular in taking their medication after variable periods of time and consequently suffer partial or complete relapse of the toxic thyroid state. Second, security as far as protecting the patient against a possible attack of agranulocytosis, depends upon the early recognition and heeding of symptoms such as fever, sore throat, dermatitis, and other toxic manifestations. When such symptoms do occur, as has been emphasized, then the patient should at once consult a physician promptly and have a white blood cell count and differential count done. In no other way can agranulocytosis be detected and the life-saving penicillin therapy instituted early in the course of the disease. If a patient is not sufficiently dependable to coöperate, it is recommended, for the reasons given above, that a thyroidectomy be performed after thiouracil has been administered preoperatively under close observation for a sufficient period of time to secure the maximum fall in the basal metabolic rate.

Cosmetic Effect. The occasional large toxic goiter which presents an undesirable

cosmetic defect should, of course, be removed surgically. This is not in any way a complication of thiouracil therapy. The removal of such large goiters, however, is attended by a higher morbidity than when the gland is smaller. It is all the more important, therefore, to reduce the toxicity as far as possible preoperatively. In our opinion this can best be accomplished with thiouracil.

Reactions Not Usually Requiring the Discontinuance of Thiouracil. A consideration of the published reports dealing with the undesirable effects of this drug leads one to suspect that possibly all of the reported reactions are not due to thiouracil. For example, in 1 small series a high incidence of a certain type of reaction occurred, such as jaundice, while in other large series this complication has never been present. One of our own patients developed fever and diarrhea which was at first attributed to thiouracil. The symptoms disappeared promptly, however, following removal of a fecal impaction.

Dermatitis. This usually is of a maculopapular variety, sometimes morbilliform, and occasionally urticarial in type. There was only 1 case of dermatitis in our series of 80 patients which we thought undoubtedly was due to thiouracil. The rash disappeared when the drug was discontinued and did not reappear when it was resumed. This reaction was of an acneform variety with intense pruritus and accompanied by leukopenia. Three of our patients developed a dermatitis which was thought to be due to thiouracil but disappeared when only phenobarbital was withheld. It is possible that some of the reported episodes of dermatitis have actually been due to various hypnotic drugs, such as the barbiturates, administered coincidentally with thiouracil. Williams and Clute¹⁹ report 4 cases of dermatitis in 152 patients (2.6 %), Gargill and Lesse⁵ none in 43, and Lahey and Bartels¹⁰ 4 in 190 (2.1 %). Most authors agree that this reaction may be avoided when the drug is resumed, especially if it is given in smaller doses.

Leg Edema. Edema of the legs has been reported to occur by Williams and Clute¹⁹ in an incidence of 3.9%. Such a complication is not mentioned as having been present in the series of 190 patients reported by Lahey and Bartels,¹⁰ nor has it been observed in our patients.

Jaundice. This complication has been reported as developing in 2 of 43 patients of Gargill and Lesses (4.6%)⁵ but not in the large series of Lahey and Bartels¹⁰ or in that of Williams and Clute.¹⁹ Other authors with smaller series, however, have also noted this complication. It has not been observed in our patients. Though Gargill and Lesses have made a very thorough study of their cases of jaundice, apparently there are no reports of recurrence of the jaundice following a resumption of the thiouracil therapy.

The Goitrogenic Effect of Thiouracil. A majority of authors agree that thiouracil does not produce significant enlargement of the thyroid gland grossly in man. Astwood² states that in some of his patients a definite goitrogenic effect was observed. The point is unsettled because no accurate means of measuring thyroid size has been employed. The gland often appears to diminish in bulk because it may soften during thiouracil administration and consequently blend more readily with the normal neck contour. It is logical to assume that the lack of goitrogenic effect in humans is due to (1) smaller dosage of thiouracil on body weight basis than that used in rats to produce goitrogenic effect, and (2) hypothyroidism is uncommonly produced in humans and, therefore, the stimulus to thyrotropic hormone production is not as great as in the animal with hypothyroidism induced by the drug.²

Production or Accentuation of Exophthalmos. In our experience there has been no higher incidence of progressive exophthalmos in patients treated with thiouracil than in those in whom a thyroidectomy was performed. Some authors believe that the slower lowering of the B.M.R., as in thiouracil treatment, is less liable to induce progressive exophthalmos than

surgical therapy. At any rate, we are now using thiouracil plus desiccated thyroid to treat malignant exophthalmos.

Other Reactions. These include "allergic" arthritis and cervical and salivary gland adenopathy. Adenopathy has occurred in our series only in conjunction with neutropenia. The case of generalized adenopathy reported by Gabrilove and Kert,⁴ however, did mention that the peripheral blood was normal at the time of the reaction.

Conclusions. In our experience thiouracil has proved to be a valuable drug when employed preoperatively in patients with both nodular and hyperplastic toxic goiter. It is superior to iodine when used in this way because the basal metabolic rate may be brought to zero or lower, and also because it is equally effective in both types of toxic goiter. In our experience, iodine has not been highly satisfactory in the preoperative treatment of patients with toxic adenoma, and also in many cases with the exophthalmic type, especially when it has been previously administered, or possibly when the patients have ingested iodized salt over a long period of time. In only 1 patient in our group of 80 has the B.M.R. failed to fall to below zero following the administration of the drug. In this patient, 18 years of age, the lowest B.M.R. was +9%. The importance of the reduction of the basal metabolic rate to normal before thyroidectomy is performed is recognized by all physicians who have had experience in this field; it is especially emphasized by the observation that when such patients are "iodine resistant" the operative mortality rate is 24 times higher than in the patients in whom a satisfactory result has been attained by the use of iodine before thyroidectomy was performed.¹⁴ Furthermore, it is recognized that thiouracil may prove, in certain instances, to be the treatment of choice in patients with toxic goiter. Further conclusions concerning this will be presented in a subsequent paper now in preparation.*

* This paper has since been published in J. Am. Med. Assn., 131, 735, 1946.

The most serious objection to the use of the drug is the occasional occurrence of agranulocytosis which may terminate fatally. It is our belief, however, that if this complication is kept in mind and detected at the earliest possible moment, then prompt discontinuance of the drug, and institution of penicillin therapy should avert a fatal issue in all instances. Nevertheless, it must always be recognized as a dangerous complication, and the patient should be instructed to report to a physician for a blood examination when even the slightest warning symptoms appear.

Summary. 1. Ten fatal cases of agranulocytosis occurring during the administration of thiouracil have been collected from the literature and by personal communications. They reveal a common pattern when analyzed which gives definite clues to follow in the prevention of fatalities due to this complication.

2. The most satisfactory method of preventing fatalities from agranulocytosis

is to warn the patient who is taking thiouracil to discontinue the drug promptly following the appearance of fever with or without sore throat, skin rash or adenopathy. If a white blood cell count and estimation of the neutrophil percentage shows a significant depression of the cells, then penicillin should be administered immediately until such time as the blood count becomes normal again.

3. Thiouracil therapy may then be resumed but it should be recognized that there is a definite risk of recurrence of this agranulocytic reaction.

4. Other complications requiring thyroidectomy ultimately are febrile reactions, relative thiouracil resistance, nodular thyroid, large goiter, and failure of the patient to cooperate.

5. The remaining complications occurring during thiouracil administration ordinarily do not require more than temporary cessation of the drug.

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STUDIES ON THE PENETRATION OF SULFONAMIDES INTO THE SKIN

III. PENETRATION OF SULFANILAMIDE AND SULFATHIAZOLE INTO INTACT AND INJURED SKIN OF GUINEA PIGS FROM VARIOUS VEHICLES, INCLUDING POLYETHYLENE GLYCOLS ("CARBOWAX")*

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In the first 2 papers of this series,^{36,37} the effect of time of application of water-in-oil (W/O) and oil-in-water (O/W), representative ointments upon penetration of different concentrations of sulfonamides, was studied in intact skin of guinea pigs and humans. Concentration of sulfonamide had little effect, whereas longer times of application increased penetration. A few experiments were reported on penetration into injured skin. The more soluble sodium sulfacetimide penetrated more than sulfanilamide (SNA), sulfathiazole (SAT) or sulfadiazine (SAD). Wet packs with a detergent gave greater penetration than any of the ointment bases studied. In injured skin, preliminary studies showed that the concentration of sulfonamide as well as time of application determined the extent of penetration.

Since the first 2 papers of this series appeared, several workers have reported on penetration of sulfonamides. Many workers have studied sulfonamide liberation and diffusion from various ointments, by utilizing the cup test against bacterial plate cultures, or qualitative or quantitative determination of the concentration of liberated sulfonamides in an aqueous phase.^{17,18,21} These methods are open to criticism if not compared directly with *in vivo* experiments, particularly when applied to skin, since the skin barriers are multiphase systems and are not strictly comparable with aqueous solutions.

Other investigators have used blood levels as a measure of the penetration of topically applied sulfonamides.^{24,47} This technique does not reveal local concentrations attained by sulfonamides liberated from various vehicles, and at best relates relative rates of penetration. If compared with local tissue concentrations, however, it yields valuable information.

Various new methods have been used to investigate the penetration of drugs. Johnston and Lee¹⁷ suggest radioactive tracer compounds to follow skin absorption. MacKee, Herrmann, Baer and Sulzberger^{21,22} introduced a method for the histochemical detection of sulfonamides and other drugs in the skin after topical application. The method involves gaseous fixation to avoid displacement of drugs in tissues by diffusion. The method identifies loci of penetration but does not permit quantitative assessment of the amount of penetration although it does permit qualitative estimates. Later the same workers,²³ using such techniques, described the effects of surface active agents, vehicle effect, solubilizers and coupling agents on the course of penetration, and estimated the degree of penetration of sulfonamides into the intact skin.

Helander,^{13,14} by freezing-lyophilization fixation and fluorescent microscopy of tissue sections, was able to identify loci of penetration of sulfonamides into tissues.‡ These techniques do not quantitatively

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‡ Miescher²⁵ described similar techniques for identifying penetration of substances other than sulfonamides into intact skins of guinea pigs.

measure the amount of penetration, although they yield useful information on the course of penetration and a qualitative measure of its extent.

Woodard *et al.*^{32,42} on the other hand, evaluated the penetration of sulfonamides into intact rabbit skin by direct chemical analysis of skin biopsies, as well as by blood and urine analysis. The effects of time of application, concentration of sulfonamide, vehicle and surface active agents were studied. Glycol bases as sulfonamide solvents were superior to the usual W/O, O/W, fatty or jelly bases. In agreement with the first 2 reports of the present series,^{36,37} they found that concentration had little effect (5% SAT gave as much penetration as 20%) and that penetration increased with time. Studies on injured skin were not reported.

Much has been written on penetration of sulfonamides into wounds. Hawking^{10,11} studied penetration into adjacent tissues and blood of crystalline SNA and SAT locally implanted in wounds, as well as comparing *in vitro* with *in vivo* studies of penetration of SNA from various ointment bases.⁹ Waud⁴¹ determined the concentrations of SAT at different levels of muscle underlying the skin of rabbits, when various concentrations of SAT were implanted subcutaneously in different bases for various times. Penetration was found to be dependent upon time, concentration and the type of base. Water-soluble or O/W emulsion bases gave better penetration than W/O or oily bases. In no event did the tissue concentration exceed 5 to 6 mg. per 100 gm., and the concentrations of SAT in the tissue beyond 2 to 3 mm. in the muscle were less than if the SAT were administered orally. This is in agreement with Hawking,¹¹ who found that SAT does not penetrate mus-

cle tissue more than 2 to 3 mm. before it is swept away by blood. When the circulation is impaired, as in gangrene, the local tissue concentrations may reach much greater levels, as shown by Reed and Orr.²⁸

Wound exudates contain nearly the same sulfonamide concentration as blood, after oral administration;³¹ thus the levels are necessarily lower than can be obtained by local application if the blood does not sweep away the drug.

The present study had for its aim an extension of the studies on penetration of sulfonamides into injured skin from various vehicles, noting particularly the effects of concentration, time of application and the nature of the vehicle. Emphasis has been made of the polyethylene glycols ("Carbowaxes") as vehicles in various forms because of their low toxicity* and because of their high solvent powers for the sulfonamides. Yonkman *et al.*^{43,44,45,46} describe propylene glycol as a non-toxic solvent for high concentrations of sulfonamides, and since then this glycol has been used in surgery.³ The use of propylene glycol led to the study of the less toxic higher glycols. Appleby² dissolved high concentrations of SNA in propylene glycol and in a mixture of propylene glycol and triethylene glycol, and used such solutions, along with urea, in veterinary surgery. At the present time, the polyglycols such as the polyethylene glycols ("Carbowaxes"⁴⁵) are finding more use in dermal cosmetic preparations⁴⁵ and as ointment bases.^{18,29} The solubility of sulfonamides in these substances is higher than in any other non-toxic excipient yet described. SAT and SNA make solutions of 15 to 25% in these water-soluble "waxes."[†] Moreover the addition of either SNA or SAT in the form of aqueous-

* The toxicity of glycols decrease with increased molecular weight,⁴⁷ and the glycols, particularly the polyglycols have a very low toxicity.^{48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100} The higher glycol polymers ("Carbowax 1500, 1540, 4000")¹ have nearly no local toxicity on intact skin, and low irritating qualities on mucosa and the eye,^{44,45} and do not impair epithelization of wounds.^{4,11}

† Approximate solubilities of SNA and SAT in Carbowaxes at room temperature (data supplied by the Westwood Pharmacal Corp.):

Solvent		Cbwx-1500					Cbwx-4000			
Solvent concn., % (aq. sol.)		100	90	80	65	50	50	70	65	50
% SAT	21				7	24	16	11	6
% SNA	15	20	17	8	4	14	10	7	4

solutions in 50% Carbowax 4000⁵ effects higher concentrations of these drugs in blood serum than does the addition of either SNA or SAT in the form of powders, as well as afford greater bacteriostatic action against *S. aureus*.*

Methods. 1. The techniques of applying the ointments to normal and abraded† skin of guinea pigs held in confining cages,‡ and the method of analysis of skin biopsies, was described in the first 2 papers of this series.^{36, 37} The work of Woodard *et al.*,⁴² already described in the introduction above, included a technique of biopsy analysis essentially similar to our previously reported methods, as confirmed by personal communication with Dr. R. B. Smith, Jr.,³² of the Food and Drug Administration.

2. *Sulfonamides.* Only SNA and SAT were studied, in concentrations ranging from 1 to 30%.

3. *Time of Application.* From 0 to 6 hours.

4. *Site of Application.* Since there were large individual variations from animal to animal, due presumably to skin and abrasion* differences, the data were considered more significant when one or more variable was appropriately controlled in the same animal. If, for example, SAT concentration was the variable, this variable was controlled on the same animal, keeping vehicle and time of application, as well as the condition of the skin a constant. If 10% SAT gave more penetration than 5%, the number of times it exceeded 5% was considered a better basis for comparison than the actual tissue concentrations found, if comparisons were to be made between different animals and between different experiments.

5. *Biopsy Analysis.* The method of biopsy has been described previously.^{36, 37} Usually 2 to 4 animals were used per experiment. At least triplicate tissue aliquots were analyzed from each biopsy, and in some cases 6 to 8 per biopsy. The experiments

were repeated at least once, and in some cases several times, as indicated by the columns "Expt. No." and "No. of analyses" in the tables.

6. *Bases and Reagents.* In the first 2 papers of this series, O/W and W/O bases were compared, and in a few cases, wet packs with detergents. In the present report, the bases studied are listed below:

"Hydrosorb" (Abbott): W/O base consisting of oleic acid, white petrolatum and oleic acid esters and amides of diethanolamine. The sulfonamides were incorporated by tile and spatula.

Base 104, W/O: "Aquaphor" (Duke), a W/O base consisting of alcohols and cholesterol esters in petrolatum, 45.5%; Carbowax 4000,⁵ a polyethylene glycol of approximate mol. wt. of 3600, a water-soluble waxy solid, 45.5%; water, 9%. Heated and emulsified as cooled. Sulfonamides dissolved in the Carbowax-water phase by heat before emulsifying with Aquaphor.

Carbowax 50, 50%: 50% aqueous Carbowax 4000, containing desired concentration of sulfonamide, dissolved with heat. A solution, applied as wet packs, wetting every 30 minutes or so. Cover packs with waxed paper.

Carbowax 1500, 50%: Same as above, but using Carbowax 1500 instead of 4000.

Carbowax 70-30: Carbowax 4000, 70%; Carbowax 1500, 30%. Carbowax 1500 is a mixture of polyethylene glycols of an average mol. wt. of 1250, containing equal parts of Carbowax 300 (hexaethylene glycol) and Carbowax 1540.⁵ It is a soft, translucent substance with the consistency of petrolatum. Sulfonamides dissolved with heat.

Carbowax 80-20: Carbowax 4000, 80%; Carbowax 1500, 20%. Too stiff alone, but plasticized by addition of SAT.

Carbowax 19-75: Carbowax 4000, 75%; Carbowax 1500, 19%; water, 6%.

Base 121 (O/W): Carbowax 4000, 55%; white petrolatum, 30%; "Arlacel-C" (Atlas

* Summary of laboratory reports supplied by the Westwood Pharmacal Corporation.

† As described previously, the depilated skin was uniformly and severely abraded with flooring sandpaper. Scalpel scratches and cuts, as well as abrasion by sandpaper, are difficult to reproduce. Recent attempts indicate that it is feasible to produce a uniformly deep denuded epithelium by the use of a 4 inch Padgett Dermatome,²⁷ used in obtaining donor grafts (Kansas City Assemblage Co., 609 E. 17th St., Kansas City, Missouri). The Dermatome first is covered with adhesive tape of the width desired for the denuded area, then covered with Dermatome cement. The skin is clipped, shaved and depilated with a paste made by mixing water with 20 parts starch, 20 parts zinc oxide and 60 parts of strontium sulfide. The skin also is covered with a layer of Dermatome cement. The Dermatome knife should be made of hard steel and must be very sharp.

‡ Other restraining devices, for long duration experiments on small animals have been described.^{1, 22}

§ "Westhiazole" base.

Powder Co.; an emulsifier, one of the long chain esters of the hexitol inner ethers), 5%; water, 10%. Emulsified at 55° C. Sulfonamide dissolved in Carbowax phase.

Base 121A: Same as 121, but increase Carbowax to 58% and decrease water to 7%.

Base 101 (O/W): Carbowax 4000, 19%; Carbowax 1500, 29%; white petrolatum, 29%; stearic acid, 19%; sodium lauryl sulfate, 2%; water, 2%. Sulfonamide dissolved in Carbowax. Stearic acid dissolved in Carbowax. Water plus lauryl sulfate emulsified with petrolatum plus Carbowax phase, while warm.

Base 102 (O/W): Carbowax 4000, 16%; Carbowax 1500, 25%; white petrolatum, 38%; stearic acid, 16%; sodium lauryl sulfate, 2%; water, 3%. Handled same as 101.

Base B (O/W): (Similar to Base B described in first paper of this series.) Liquid petrolatum, 41%; corn oil, 2%; triethanolamine, 1%; stearic acid, 3%; cetyl alcohol, 2%; water, 51%.

5% "Methocel" Gel: (5% Methocel (Dow) in water. Methocel is methyl cellulose. The type used was Dow 4000CPS.) 50 cc. water brought to a boil in beaker. Add 2.5 gm. Methocel and wet thoroughly. Place beaker in brined iced water and cool, stirring carefully to avoid bubble formation. Just before mixture gels, add 5 cc. Ehrlich's reagent* and mix. Pour ca. 18 cc into large test tube and gel in iced water. A stiff, water-transparent gel results which turns yellow on addition of sulfonamides.

Locke's agar with Ehrlich's reagent: (Used by Lum²⁰ for sulfonamide diffusion experiments.) Agar 40 gm.; NaCl, 9 gm.; KCl, 0.2 gm.; CaCl₂, 0.2 gm.; water to 1 liter. To each 100 cc. liquid agar add 1.25 cc. Ehrlich's reagent just before pouring into tubes.

7. *In Vitro* Diffusion Experiments. Effects of time of diffusion, sulfonamide concentration, and vehicles. SAT, 1%, 5%, 10% and 30% in Aquaphor, Base 121, Base 104 and Carbowax 70-30, and as a pure powder or 10% aq. suspension of powdered or "Micro-crystalline" SAT,† were studied, with respect to rate and relative intensity of diffusion, in 4% Locke's agar with Ehrlich's reagent. The yellow color with Ehrlich's reagent, when viewed through a blue filter appears

as a bright orange or red. A Wratten filter combination of maximum absorption of 4300 to 4700 Å was used, and a light source concentrated through a slit and lens system illuminated the tubes from behind. The agar or Methocel gels were contained in 6 inch test-tubes and the sulfonamide preparations placed on top. The tubes were incubated at 37° C. and observed daily for 6 to 8 days. In a second experiment, instead of using agar (not transparent, slightly yellow), a water-transparent Methocel gel was used. A wet cotton disk diffusion barrier was placed between the gel and the sulfonamide preparation. SAT, 1%, 5% and 10%, was incorporated into Carbowax 70-30, Base B, Base 104, Aquaphor, and as a saturated aqueous solution.

Results. 1. *Effect of Skin Injury on Penetration.* Table 1 and Figure 1 summarize the results of 6 experiments on 18 guinea pigs, in which 207 biopsy analyses were performed. SNA and SAT, 10 and 20%, suspended in Hydrosorb and dissolved in Carbowax 70-30 or Carbowax 50, were applied for 4 hours to normal and abraded areas on the same animals.

Abrasion increased the penetration of both SNA and SAT from Hydrosorb 1 to 4 times, whereas it increased penetration from the Carbowax mixtures 3½ to 9 times. In every case the Carbowax increased the abrasion effect more than Hydrosorb. The average skin concentration ranges were 7 to 18 mg. per 100 mg. skin for normal skin and 17 to 85 mg. per 100 gm. for abraded skin.

2. *Effect of Time of Application.* In all cases abraded areas were used. SAT (1, 5, 10 and 30%) was used in Base 104. Only 1 concentration of SAT was used on any 1 animal, and was applied for 1 and for 6 hours to this animal. Table 2 and Figure 2 summarize the results.

It is seen that for Base 104, 6 hours application gives only av. 1.4 times greater penetration than 1 hours application, and that concentration has little or no effect on this difference. Table 3, dis-

* p-Dimethylaminobenzaldehyde (Eastman), 1.57 gm.; 10% HCl (U.S.P. 23.6 cc. conc. HCl in 100 cc.), 20 cc.; water, to 100 cc.

† Smith, Kline & French Laboratories, Philadelphia.

cussed below, illustrates the same thing but in a different mode of expression.

3. *Effect of Concentration of Sulfonamide.* SAT in Base 104 was applied for 1 or for 6 hours to abraded skin. The concentrations compared in any 1 animal were 30 versus 1%, 30 versus 5% and 30 versus 10%. The results are summarized in Table 3 and Figure 3.

It is seen that only 1% SAT gives markedly less skin concentrations than 30%. The tissue concentration ranges increase with concentration but are above an average of 100 mg. per 100 gm. with 10% SAT even within 1 hour. It is concluded that although concentration increases penetration, 5% SAT is as effective bacteriostatically as 30%. It is

TABLE 1.—EFFECT OF SKIN INJURY ON PENETRATION OF SULFONAMIDES FROM HYDROSORB AND CARBOWAX

Exp. No.	No. G. pigs used	No. analyses	Sulf. used and concn.	Base applied	Hrs. applied	Variable giving greatest penetr.	Av. of times greater	Tissue sulf. concn. (mg./100 gm.)		
								Range	Av.	
21	3	18	SNA, 10%	Hydrosorb	4	Abrased	1.1	Normal	7-20	11.5
		18	SNA, 10%	Carbowax 70-30	4	Abrased	3.7	Abrased	10 26	17.0
								Normal	5-14	8.0
								Abrased	18 38	26.0
22, 23	6	18	SNA, 10%	Hydrosorb	4	Abrased	2.1	Normal	11-15	12.5
		35	SNA, 10%	Carbowax 1500 50%	4	Abrased	7.5	Abrased	23-33	26.5
								Normal	6-15	11.0
								Abrased	31-200	85.0
24, 26	7	39	SNA, 20%	Hydrosorb	4	Abrased	1.5	Normal	16-22	18.0
		39	SNA, 20%	Carbowax 70 30	4	Abrased	3.5	Abrased	22-59	30.0
								Normal	13 26	18.0
								Abrased	40-140	71.0
25	2	20	SAT, 20%	Hydrosorb	4	Abrased	3.9	Normal	7-9	8.0
		20	SAT, 20%	Carbowax 70-30	4	Abrased	9.4	Abrased	21-46	33.0
								Normal	6-9	7.0
								Abrased	40-100	70.0

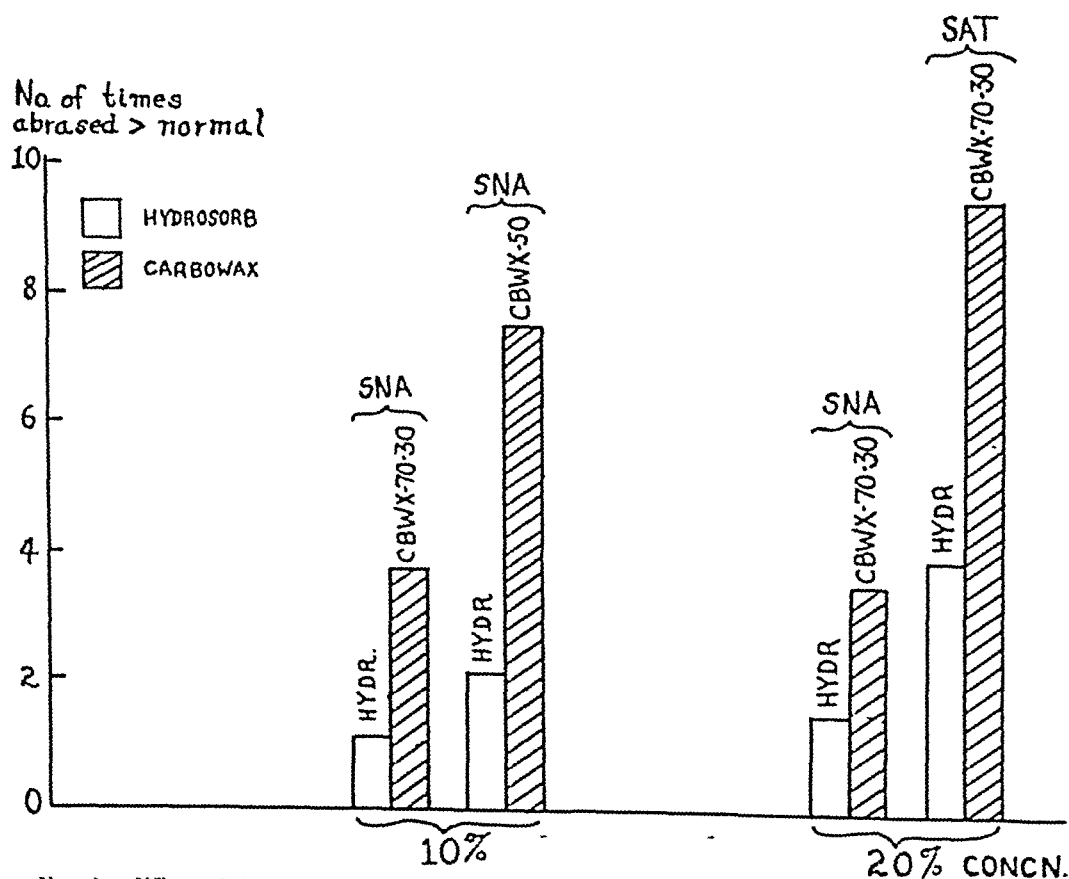


FIG. 1. - Effect of skin injury on penetration of sulfonamides from Hydrosorb and Carbowax.

interesting that Woodard *et al.*⁴² found that 5% SAT gave as much penetration as 20% in intact skin of rabbits, in agreement with previous reports,^{36,37} which showed no greater effect with 10% SNA than with 1% in intact skin of guinea pigs and humans.

Table 4 compares the penetration of 1 and 30% SAT from other bases than Base 104.

4. *Effects of Bases.* (a) *Normal Skin.* 20% SNA in Hydrosorb and in Carbowax

70-30 was applied for 2 hours to the same animal. Table 5 summarizes the results.

It is seen that there is no difference in penetration into normal skin from Carbowax than from Hydrosorb, within 2 hours. From the results previously reported,^{36,37} it was shown that penetration of 10% SNA from Hydrosorb into intact guinea pig skin was greater after 48 hours than after 24 hours. Presumably in intact skin, 2 hours application does not suffice to cause vehicle differences. This may ex-

TABLE 2.—EFFECT OF TIME OF APPLICATION ON PENETRATION OF SAT INTO INJURED SKIN FROM BASE 104

Exp. No.	No. G. pigs used	No. analyses	SAT. concn.	Variable giving greatest penetr.	Av. No. of times greater	Tissue sulf. concn. (mg./100 gm.)		
						Range		Av.
38, 39	7	42	1%	6 hrs.	1.5	1 hr. 13-38		25
						6 hrs. 19-74		33
40, 41	8	45	5%	6 hrs.	1.9	1 hr. 15-186		65
						6 hrs. 29-286		122
42, 43	7	42	10%	6 hrs.	0.8	1 hr. 53-208		110
						6 hrs. 28-413		171
34, 43	35	209	30%	6 hrs.	1.3	1 hr. 37-465		174
						6 hrs. 49-815		271

Variables compared: 6 hrs. application vs. 1 hr.

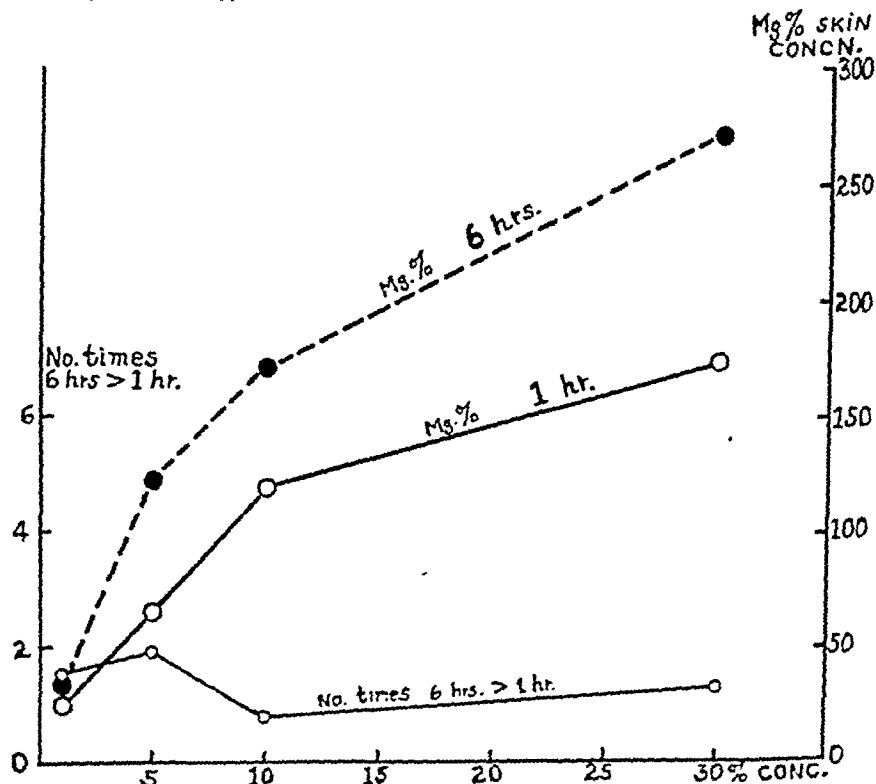


FIG. 2 —Effect of time of application on penetration of SAT into injured skin from Base 104.

plain the difference between the present results and those previously reported,^{36,37} as well as those described by Woodard *et al.*,⁴² who found glycol bases to give greater penetration than all other bases they tested. Their application times were extended up to 24 hours, as compared with 2 hours in the present study.

(b) *Injured Skin.* 10 to 30% SNA in Hydrosorb, Base 104 or Carbowax 70-30 was applied for 1 to 6 hours to abraded skin. In any 1 animal, all variables were kept constant except 2 different bases. Table 6 summarizes the results. Base 104, containing Carbowax, gave greater penetration than Hydrosorb, and

TABLE 3.—EFFECT OF CONCENTRATION ON PENETRATION OF SAT INTO INJURED SKIN FROM BASE 104

Exp. No.	Variables compared	No. G. pigs used	No. analyses	Hrs. applied	Variable giving greatest penetr.	Av. No. of times greater	Tissue sulf. concn. (mg./100 gm.)		
							Range		Av.
38, 39	1 vs. 30%	7	42	1	30%	8.8	1% 30%	13 94 38 350	25 208
35, 39	1 vs. 30%	7	42	6	30%	9.7	1% 30%	19 219 74 685	33 348
40, 41	5 vs. 30%	8	33	1	30%	1.9	5% 30%	15 37 186 418	65 155
40, 41	5 vs. 30%	8	29	6	30%	1.6	5% 30%	29 78 286 588	122 184
42, 43	10 vs. 30%	7	42	1	30%	0.8	10% 30%	53 73 208 360	119 152
42 46	10 vs. 30%	20	111	6	30%	1.5	10% 30%	13 41 413 550	114 230

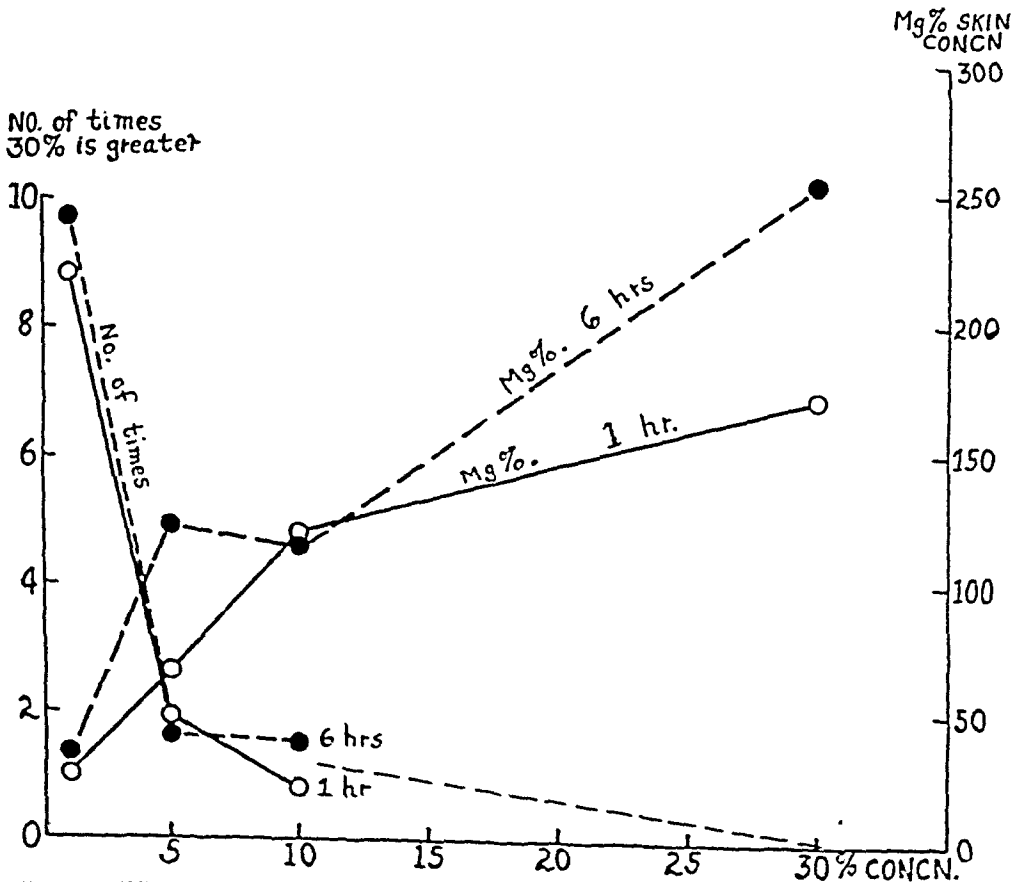


FIG. 3.—Effect of concentration on penetration of SAT into injured skin from Base 104.

pure Carbowax gave greater penetration than either Base 104 or Hydrosorb. Thus, as the concentration of Carbowax increased, the penetration increased.

5. *In vitro* Diffusion Experiments. Figures 4 and 5 depict the results of the diffusion of SAT into agar and into Methocel gel.

The results show: (1) The rate and amount of diffusion of SAT from the different preparations was as follows: Car-

bowax bases > Carbowax—grease bases > aqueous solutions, powders or O/W bases > W/O bases (Aquaphor, Hydrosorb). (2) The rate and amount of diffusion of SAT from Carbowax alone, independent of concentration, is due to Carbowax itself diffusing and carrying dissolved SAT with it. This is indicated by the fact that color standards of SAT showed much less intense color for saturated aqueous solutions of SAT than the diffusion fronts in the

TABLE 4.—EFFECT OF CONCENTRATION ON PENETRATION OF SAT INTO INJURED SKIN FROM VARIOUS BASES

Exp. No.	Variables compared	No. G. pigs	No. analyses	Base	Hrs. applied	Variable giving greatest penetr.	Av. No. of times greater	Tissue sulf. concn. (mg./100 gm.)	
								Range	Av.
29	1 rs. 30%	2	12	Carbowax 19-75	2	30%	6.0	1%, 22-55 30%, 144-330	40 237
30	1 rs. 30%	2	12	Carbowax 70-30	2	30%	8.5	1%, 13-18 30%, 106-160	16 135
31	1 rs. 30%	2	6	Carbowax 80-20	1	30%	23.2	1%, 21-27 30%, 330-790	23 560
32	1 rs. 20%	2	12	101	1	20%	26.0	1%, 11-13 20%, 210-400	12 305
			6	101	6	20%	29.8	1%, 29-31 20%, 880-900	39 893
33	1 rs. 20%	3	18	102	1	30%	3.6	1%, 35-60 30%, 155-200	48 169
			18	102	6	30%	14.0	1%, 35-51 30%, 120-860	48 617

TABLE 5.—EFFECT OF BASES ON PENETRATION OF SNA INTO NORMAL SKIN

Exp. No.	No. G. pigs	No. analysis	Base giving greatest penetr.	Av. No. times greater	Tissue sulf. concn. (mg./100 gm.)	
					Range	Av.
12, 15, 16, 18	10	61	Carbowax, 70-30	0.7	Carbowax, 3-17 Hydrocarb, 2-24	10.5 7.5
13, 18, 24, 26	12	80	Hydro-sorb	0.4	Carbowax, 1-26 Hydro-sorb, 3-22	13.0 14.0

Bases compared: Carbowax 70-30, hydrosorb.

TABLE 6.—EFFECT OF BASES ON PENETRATION OF SULFONAMIDES INTO INJURED SKIN

Exp. No.	Bases compared	No. G. pigs	No. analyses	Sulf. and concn.	Hrs. applied	Base giving greatest penetr.	Av. No. times greater	Tissue sulf. concn. (mg./100 gm.)	
								Range	Av.
21	Hydro-sorb Carbowax, 70-30	3	18	SNA 10%	4	Carbowax	1.5	Carbowax, 19-38 Hydro-sorb, 10-26	26 17
23	Hydro-sorb Carbowax, 50-50	3	18	SNA 10%	4	Carbowax	1.5	Carbowax, 31-51 Hydro-sorb, 23-50	40 26
16, 17	Hydro-sorb Carbowax, 70-30	4	22	SNA 20%	2	Carbowax	4.7	Carbowax, 117-256 Hydro-sorb, 23-46	161 33
14, 20, 24, 26, 27	Hydro-sorb Carbowax, 70-30	14	86	SNA 20%	4	Carbowax	2.9	Carbowax, 34-202 Hydro-sorb, 19-83	87 32
25, 28	Hydro-sorb Carbowax, 70-30	5	38	SAT 20%	4	Carbowax	2.0	Carbowax, 40-100 Hydro-sorb, 21-46	67 34
44	Base 104 Carbowax, 70-30	5	30	SAT 10%	6	Carbowax	3.2	Carbowax, 65-278 104, 17-385	153 104
35, 36, 37	Hydro-sorb Base 104	10	60	SAT 30%	1	Base 104	2.5	104, 73-370 Hydro-sorb, 45-125	180 75
35, 36, 37	Hydro-sorb Base 104	10	60	SAT 30%	6	Base 104	2.1	104, 68-815 Hydro-sorb, 76-200	314 124

tubes with Carbowax-containing bases, and by the fact that the diffusion from 30% SAT is slightly less than from 1, 5 or 10% SAT, due probably to the fact that there is relatively less Carbowax vehicle to carry the SAT. The diffusion of SAT from Aquaphor and Hydrosorb was bacteriostatistically insignificant (less than 0.5 mg. per 100 cc.). (3) In agar the concentrations of SAT in Carbowax-grease base mixtures (Base 104, 121, 121A) determine the rate and amount of

diffusion up to 10%, at which concentration the values are the same as for Carbowax alone. This probably is explained by the fact that since Carbowax is present in Base 104, 121 and 121A in the amount of 45 to 55%, increasing the concentration of SAT in these bases causes more SAT to diffuse into the agar in the Carbowax as a carrier. Carbowax diffuses faster than SAT, but the diffusing Carbowax concentration becomes lower, until after 48 hours the SAT carried in the dif-

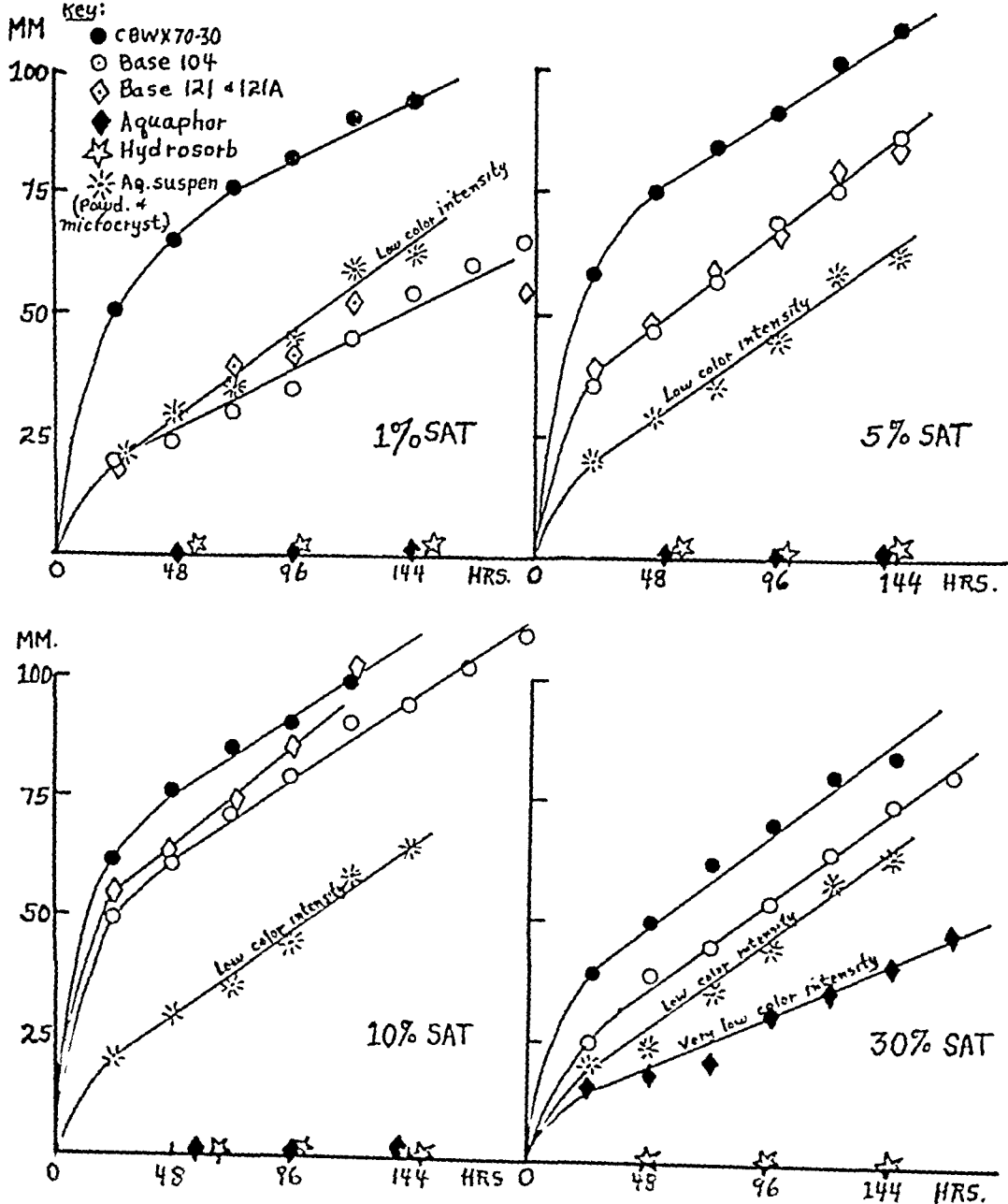


Fig. 4.--Rate of diffusion of SAT in agar from various bases.

fusing Carbowax becomes effectually as low in concentration as that diffusing from aqueous solutions. Hence the slopes of all the curves are similar after 48 hours.* In Methocel, diffusion from O/W Base B and Carbowax-grease Base 104 is the same at 1 and 5%, but at 10% the diffusion from Base 104 is greater than an O/W base. In Methocel, any addition of grease to Carbowax impairs SAT diffu-

sion. The Methocel permits Carbowax to diffuse carrying SAT with it at a constant rate, hence the more Carbowax, the greater the rate throughout the period of observation, unlike agar, which showed this only during the first 48 hours.

Cup plate tests with *S. aureus* showed the same thing as the above diffusion experiments, with certain exceptions. SAT (1, 5, 10 and 30%) in Carbowax 70-30,

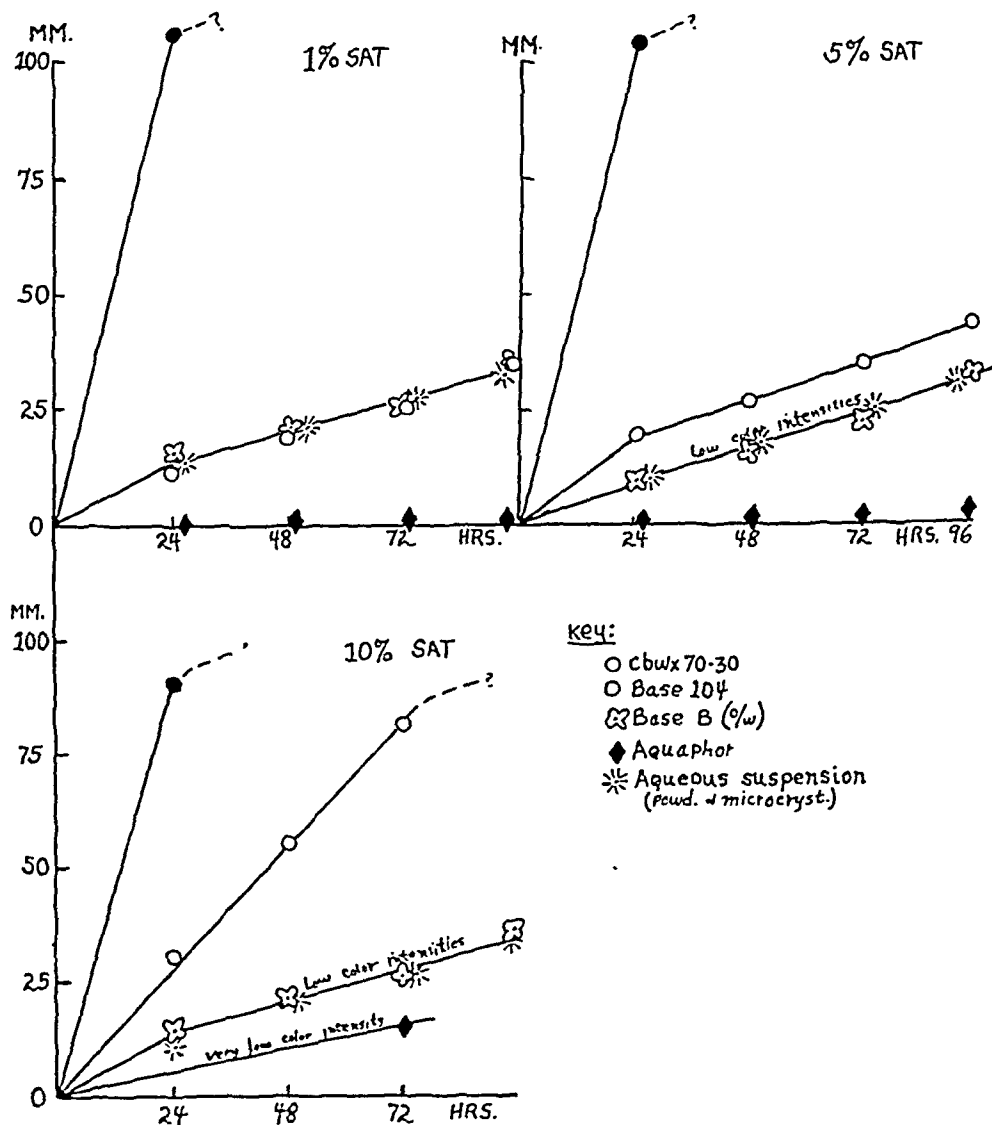


FIG. 5.—Rate of diffusion of SAT into Methocel gel from various bases.

* The exception in the case of Aquaphor containing 30% SAT can be explained by uncertainty of the readings, since the color intensities were very low.

Base 104, Base 121 and Hydrosorb were compared with respect to inhibition zones. Base 104, Base 121 and Carbowax 70-30 gave quite similar zones, whereas Hydrosorb gave negligible inhibition, as could be predicted from the agar diffusion experiments. The fact that the amount of Carbowax in a base determined the amount of diffusion in the gel experiments and *in vivo*, while it seemed to have little effect in the cup tests, illustrates one danger of application of results of agar cup plate tests to *in vivo* conditions, especially skin. Fuller *et al.*⁹ showed that *in vitro* diffusion experiments gave similar results as muscle diffusion experiments, but this does not mean that skin would be similar.

Discussion. Whether O/W or W/O type, the base and concentration of sulfonamide has little effect on penetration into normal skin. Previous work^{26,27} showed the same thing, but that time of application after 1 to 2 days had some effect. Other workers have shown that surface-active substances break down the skin barriers to some extent.^{21,23,30}

In injured skin, the type of base and the concentration of sulfonamide determine the penetration. Carbowax is an excellent vehicle for sulfonamides because of its low toxicity, great solvent powers for sulfonamides and its high penetration properties.

Penetration into injured skin probably is not significantly increased bacteriostatically from concentrations greater than 10%, as indicated from both the *in vivo* and *in vitro* experiments.

In injured skin, the type of base determines penetration. Carbowax probably gives greater penetration than aqueous solutions, O/W bases or sulfonamide powders because the base itself penetrates, carrying the sulfonamide with it, and because Carbowax-serum mixtures dissolve more sulfonamide than serum alone. Precipitation from Carbowax into serum occurs as ultrafine microcrystals, affording a further good reservoir for protracted absorption.

The local concentration of topically applied sulfonamides attained in injured skin, using appropriate vehicles, exceeds those attained in most wounds presumably because the reservoirs supply the drug faster than the blood carries it away.

In papers which will follow, the effects of various vehicles and sulfonamides on the rate of epithelization of wounds and on contaminated wounds will be described.

Summary. 1. *In vivo* experiments on guinea pigs have been performed, to determine the penetration of sulfonamides from various vehicles into intact and injured skin. In addition, *in vitro* diffusion experiments have been performed for a comparison.

2. Skin injury increased the penetration of sulfonamides from a water-in-oil base ("Hydrosorb") 1 to 4 times, whereas it increased penetration from polyethylene glycol mixtures ("Carbowaxes") up to 9 times.

3. Time of application, comparing 1 hour with 6 hours, has very little effect on penetration into abraded skin from a "Carbowax"-petrolatum base, and sulfonamide concentration has no effect upon this difference. Maximum skin levels are attained within 1 hour or less.

4. With respect to the effect of sulfonamide concentration on penetration into injured skin from a "Carbowax"-petrolatum base, it is concluded that although concentration increases penetration, the increase above 5% is not important. Ten per cent could be recommended as the maximum useful concentration.

5. In normal skin, the type of vehicle has no effect on penetration up to 2 hours application, but in injured skin pure "Carbowax" mixtures gave more penetration than "Carbowax"-petrolatum mixtures which in turn were superior to a W/O base ("Hydrosorb").

6. *In vitro* diffusion experiments showed that diffusion from various cases followed the progression, "Carbowax," > "Carbowax" plus petrolatum > aqueous solutions, powders or O/W bases > W/O bases ("Aquaphor," "Hydrosorb"). "Carbowax" dif-

fuses into gel carrying sulfonamides with it. Very little diffusion occurs from W/O bases.

7. Polyethylene glycols ("Carbowax-

es") are excellent vehicles for the sulfonamides because of their low toxicity, great solvent powers and high penetration of sulfonamides from them into injured skin.

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THE ADRENOTROPHIC, RENOTROPHIC AND CARDIOTROPHIC ACTIVITIES OF LYOPHILIZED ANTERIOR PITUITARY IN THYROIDECTOMIZED RATS

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It is now a well-recognized fact that thyroxine injected into various species will cause a proportional increase in kidney and heart size in relation to body weight.^{1,3,4,5,6} Swann¹¹ showed that the renal effect is independent of the pituitary since it occurs in hypophysectomized rats to approximately the same extent as in the intact. On the other hand, several authors^{8,9,10} have shown that crude anterior pituitary extracts can cause disproportionate but apparently normal growth of kidney tissue. This growth stimulation is presumably not associated with luteinizing activity since Dill and Isenhour² found no effect in the rabbit with doses of up to 7200 I.U. We (unpublished) have tested Prolactin* in rats in doses up to 5 mg. a day for 7 days and found it inactive. On the other hand, a potent thyrotrophic extract caused an increase in rat kidney size in the experiments of Murphy *et al.*,⁷ and Cameron and Carmichael,¹ suggesting that the renal enlargement obtained with anterior pituitary extracts was an indirect effect acting through the stimulation of thyroid hormone production. The experiments of Selye *et al.*¹⁰ leaves no doubt that thyroid hormone enhances the reno- and hepatotrophic activities of lyophilized anterior pituitary (L.A.P.). The question arises as to whether in the absence of the thyroid, L.A.P. can still increase the ratio of kidney or heart weight to body weight.

To investigate this problem the following experiments were performed.

Experimental. Three experiments were carried out. In the first, 28 male albino

rats weighing 140 to 170 gm. were thyroidectomized and divided into 2 groups. Of these, 14 were given a total of 30 mg. of L.A.P. suspended in water in 2 subcutaneous injections per day. To the other 14, the same volume of water was administered subcutaneously. The rats were killed 16 days after the first injection and the adrenals, hearts and kidneys were fixed in Suza and weighed. In Table 1, Experiment I, are given the average final body and adrenal weights for the 2 groups, and the ratios of kidney and heart weights to body weight expressed in terms of per cent, together with the respective standard errors.† The completeness of thyroidectomy was confirmed at autopsy by careful inspection of the site of operation with magnifying glasses.

It was not possible to employ as the criterion of completeness, the presence of thyroidectomy cells in the anterior pituitaries of these animals, since the abundant appearance of castration cells interfered with their recognition.

As will be seen from perusal of Table 1, Experiment I, L.A.P. caused a marked increase in body weight but no preferential increase in heart or kidney weights. There arose, however, the question as to whether L.A.P. at this dose level and in this period of time would cause a proportionate increase in kidney and heart size in normal rats of approximately this size. Consequently a second experiment was performed.

Two groups of 10 male, hooded rats, 200 to 220 gm. in weight, were thyroidectomized and castrated. Two similar

* Kindly supplied by Dr. E. Schwenk of the Schering Corporation of Bloomfield, New Jersey.

† S.E. = $\sqrt{\frac{\sum d^2}{n(n-1)}}$

Where n is the number of rats and d the deviation of the observed from the mean value.

groups were merely castrated. One group of thyroidectomized castrates and 1 group of normal castrates were injected with 30 mg. of L.A.P. subcutaneously for 16 days as in the previously described experiment. Kidneys, adrenals and hearts were fixed and weighed while completeness of thyroidectomy was confirmed at autopsy as before. The data obtained are tabulated in Table 1, Experiment II.

given, and the inevitable skin damage seen with large doses of crude protein containing extracts, could account for the absence of somatic growth response. The percentage of kidney and heart of body weight was greatly increased both in the presence and in the absence of the thyroid, though in the latter case to a somewhat lesser degree. A lessened response to L.A.P. was exhibited also by the adrenals

TABLE 1.—ADRENOTROPHIC, RENOTROPHIC AND CARDIOTROPHIC ACTIVITIES OF L.A.P. IN THYROIDECTOMIZED RATS

Experiment No.	Group No.	Treatment	No. rats	Final body weight	Average organ weights		
					Adrenal mg.	% Kidney /B.W.	% Heart /B.W.
I	1	* Tr.—exp., L.A.P.	13	221 ± 12 0	49 ± 1 4	0 79 ± 0 01	0 27 ± 0 01
	2	Tr.—exp., —	14	178 ± 4 2	33 ± 1 1	0 81 ± 0 03	0 30 ± 0 01
II	1	Tr.—exp., L.A.P.	9	240 ± 7 9	57 ± 5 2	0 78 ± 0 05	0 31 ± 0 02
	2	Tr.—exp., —	8	214 ± 7 9	38 ± 1 8	0 80 ± 0 03	0 29 ± 0 02
	3	— L.A.P.	8	250 ± 7 9	74 ± 5 1	0 85 ± 0 01	0 33 ± 0 01
	4	— —	9	220 ± 6 1	37 ± 1 5	0 80 ± 0 02	0 29 ± 0 01
III	1	Tr.—exp., L.A.P.	5	215 ± 7 6	96 ± 8 1	1 07 ± 0 08	0 39 ± 0 01
	2	Tr.—exp., —	6	193 ± 6 1	37 ± 1 8	0 81 ± 0 02	0 29 ± 0 01
	3	— L.A.P.	7	220 ± 7 5	133 ± 4 7	1 29 ± 0 06	0 46 ± 0 01
	4	— —	8	216 ± 3 8	33 ± 1 2	0 85 ± 0 01	0 29 ± 0 01

* Tr.—exp. = Thyroidectomized.

Here again L.A.P. induced a marked increase in body weight in thyroidectomized rats but no increase in the proportionate weight of kidneys or heart. On the other hand there was an increase of only doubtful statistical significance in the proportionate weight of kidneys and heart in rats with intact thyroids. Thus either the dose or the duration of treatment was inadequate.

The third experiment was similar to Experiment II. It was performed on 4 groups of 10 castrated white rats, 150 to 175 gm. in weight, 2 thyroidectomized and the other 2 with intact thyroids. The dose of L.A.P. was raised to 64 mg. per day and the duration of treatment, increased to 21 days. Completeness of thyroidectomy was again checked at autopsy. The data obtained is given in Table 1, Experiment III.

In this experiment the body weight increasing activity of L.A.P. was not apparent. Perhaps at this dose the amount of adrenocorticotrophic hormone

in this and the previous experiment. This was to be expected on the basis of the findings of Selye *et al.*,¹⁰ that in normal rats thyroid hormone enhances the trophic effects of L.A.P. on kidneys, heart and adrenals.

Thus, these experiments show that in the absence of the thyroid, cardiostrophic and renostrophic effects of L.A.P. can be elicited. These responses are not due, therefore, to an indirect secretory stimulation of the thyroid gland which, through the thyroid hormone would cause kidney and heart enlargement. Nor is the presence of thyroid hormone essential to the mechanism whereby L.A.P. produces these trophic effects. It appears merely to enhance the reactions involved. It remains to be seen whether the renostrophic effect is due to adrenocorticotrophin, growth hormone, a separate principle, or a combination of the known trophic principles. Pertinent experiments are now under way.

Summary and Conclusions. The renotropic, cardiotropic and adrenotropic activities of lyophilized anterior pituitary (L.A.P.) in thyroidectomized rats were investigated.

If an adequate dose of L.A.P. is administered for a sufficiently long time, reno-

trophic and cardiotropic responses are statistically significant even in the absence of the thyroid.

Adrenals, kidneys and heart appear to be less sensitive to the trophic effects of L.A.P. in the absence than in the presence of the thyroid.

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ATABRINE AND THE ELECTROCARDIOGRAM

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A RECENT electrocardiographic study,⁵ carried out in the field and limited to Leads IV R and IV F, ascribes a decrease in the amplitude of the various electrocardiographic deflections, especially the T wave, to atabrine in the dosage of 0.3 gm. daily for 1 week. Since the common denominator in all troops in the Southwest Pacific Area was the taking of atabrine, and since this alleged effect of the drug on the electrocardiogram might be confused with that of many of the diseases (malignant tertian malaria, tsutsugamushi fever) or drugs (emetine) being investigated, it seemed desirable to determine the effect, if any, of atabrine on the electrocardiogram in a large number of individuals, both in so-called "suppressive" dosage and in the much larger "intensive" doses then being used for the first time.

Procedure. The records were reviewed of 165 patients who for one reason or another had had electrocardiograms taken during the course of the administration of atabrine in therapeutic or suppressive doses. These studies were carried out from early 1943 to August 1944, at a numbered general hospital in Queensland, Australia, and concluded in 1944 and 1945 at another numbered general hospital in British Northeast New Guinea. In addition, a planned serial electrocardiographic study was made on a group of 14 volunteer medical and medical administrative officers at the first hospital.

In all instances the tracings were taken by the usual technique, including the conventional limb leads and the apical precordial lead (Lead IV F). In 2 men in the group of patients multiple chest leads were taken in the manner of Wilson. In the serial study of officers special care was exercised to be certain that the precordial electrode was in the same position at each recording.

STUDY OF GENERAL HOSPITAL PATIENTS. The 165 ward patients ranged in age from

19 to 54 years, but the bulk of the cases fell in the 22 to 26 year old group; 137 of these patients had had malaria at intervals of from 1 to 178 days (average 37.3 days; mean 29 days) between the onset of the malarial attack and the taking of the electrocardiogram. The species diagnosis was *Plasmodium vivax* in 114 of these patients who had had malaria, *Plasmodium falciparum* in 6, and unclassified in 17. The diagnosis in the remaining 28 cases were psychoneuroses (7 cases), no disease (8 cases), question of angina pectoris (8 cases), hypertension (2 cases) and nasopharyngitis, convalescent tsutsugamushi fever and question of rheumatic fever (1 case each).

The patients fell into 3 subdivisions according to the atabrine dosage at the time the electrocardiograms were taken: (1) 12 patients on intensive dosage only, consisting of 1.2 gm. in the first 24 hours, 0.9 gm. in the second 24 hours and 0.3 gm. daily for from 2 to 4 days (see Table 1); (2) 26 patients on suppressive doses only (0.1 gm. daily); and (3) 127 patients receiving suppressive maintenance dosage following intensive priming therapy. The range of plasma atabrine levels were calculated on the basis of the report of Shannon and his group.⁶

Among the 165 patients in the group the electrocardiogram was normal in 164 cases and abnormal in 1, a patient of 24 years who had had benign tertian malaria 20 days previously. In this case the T wave in Lead CF₄ was diphasic, in Lead CF₆ inverted. Although this is an abnormal finding, no explanation could be given for it at the time of hospitalization and the patient was returned to duty.

SERIAL ELECTROCARDIOGRAPHIC STUDY. In the group of 14 medical and medical administrative officers electrocardiograms were taken before atabrine was given, after 2 weeks on suppressive dosage (0.1 gm. daily) and after the same "intensive"

dosage described above. The calculated mean atabrine level when on suppressive doses was 16 gamma per liter and when on intensive dosage from 64 to 71 gamma per liter. None of these officers showed abnormal electrocardiograms. There were minor variations in the P-R interval but this never exceeded 0.17 second and those few variations that did occur (0.01 to 0.04 second) could be attributed to variations in the heart rate. There were no significant changes in the T waves.

been a factor producing electrocardiographic effects opposite in direction to those due to atabrine, thus theoretically masking any electrocardiographic effects of the drug.

In the field study mentioned above,⁵ in addition to its effect in decreasing the amplitude of the various electrocardiographic deflections, it was considered that atabrine restores the S-T interval to the iso-electric level after it has been elevated by plasmoquine. Other electro-

TABLE 1.—THE EFFECT OF ATABRINE IN VARYING DOSE ON THE ELECTROCARDIOGRAM OF 165 GENERAL HOSPITAL PATIENTS

	No. cases	Dosage (gm.)	Duration of medication (days)	Calculated plasma atabrine level (gamma/liter)	Normal ECG	Abnormal ECG
Intensive therapeutic doses only	12	1 5-3 7	2-6 Av. 2 6	25-67	12	0
Intensive therapy with suppressive follow-up:						
Priming dose	127	1 5-3 6 Av. 1 7 Mean 1 5	4-5	16-52	126	1
Suppressive dose		0 2 twice weekly to 0 1 7 days a week	2-44 Av. 25 Mean 23			
Suppressive doses only	26	0 1 6-7 days a week	3-183 Av. 17 Mean 21	17 (mean)	26	0

Discussion. It is noteworthy that of the entire series of 179 individuals studied there were 42 who had never had malaria. None of these showed electrocardiographic changes. Each of the officers studied serially was in excellent health. Moreover, in only 2 of the 137 patients who had had malaria, were the tracings taken at the time of the acute attack of malaria. In all of the others a considerable interval had elapsed before the tracings were taken, and the patients were afebrile. As pointed out above, for the most part the electrocardiograms were made for reasons quite unrelated to malaria, such as neuro-circulatory asthenia, convalescent scrub typhus, hypertension or the like. It seems unlikely therefore that malaria might have

cardiographic studies, however, dealing with subjects on atabrine in similar (0.3 gm. daily for from 4 to 7 days^{2,3,4}) or greater (0.6 gm. daily for several days¹) dosage showed no significant effect of atabrine on the human electrocardiogram. The present experience, including 26 individuals receiving the much higher "intensive" dosage, is in agreement with the latter studies, in that again no striking electrocardiographic abnormalities were demonstrable.

Conclusions. In a series of 179 individuals atabrine in therapeutic and suppressive doses had no significant effect on the electrocardiogram. Abnormalities observed in patients receiving atabrine can be attributed to other causes.

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SEVERE ALLERGIC REACTIONS TO PENICILLIN

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ONE of the most remarkable qualities of penicillin as a therapeutic agent is its safety of administration. The lack of unpleasant or dangerous reactions has become an accepted fact. With its increasing use, however, numerous instances of allergic reactions are being encountered. Anderson,¹ in a review of the use of penicillin, stated that 2 to 5% of all patients who receive it develop urticaria, but that there is no cause as yet to revise earlier opinions regarding the drug's lack of significant toxicity for man. This has been the experience of most who have used the drug to any degree. In the main, these reactions are minor and in most instances can be ignored. There is, however, a small group of patients who react more severely, at times alarmingly, in whom discontinuance of the drug is essential. In them, dangerous situations may result if penicillin sensitivity is not recognized promptly. Such a case was reported by Price, McNairy and White.⁵ Their patient developed alarming asthma reaching critical stages following the use of penicillin.

In an experience of more than 5000 patients treated with penicillin selected from all admissions to a Station Hospital operating on Luzon during the first 9 months following the invasion, numerous minor reactions were encountered which caused no harm to the patients. However, 6 cases (0.12%) differed in that they reacted so severely that it was necessary to discontinue the drug regardless of other considerations. Four represented examples of drug allergy and 2 a delayed reaction which simulated serum sickness. It is concerning these 6 patients that this report is offered.

Opinion differs widely regarding the

nature of these reactions. In the hope that more knowledge could be gained regarding the immunologic aspects of the reactions; intracutaneous, conjunctival, patch, passive transfer and precipitin tests were performed (Table 1). In addition, 200 patients on the wards were tested by intracutaneous, conjunctival and patch methods, then given a trial dose of 50,000 units of penicillin, in an attempt to anticipate and corroborate those individuals who might react untowardly to the drug (Table 2).

Case Reports. CASE 1. A 36 year old ambulatory white male officer was admitted to the hospital on Aug. 1, 1945, for chronic recurrent lymphangitis of the left nasolabial fold approximately 3 to 4 cm. in diameter. He was afebrile, blood count and urine were normal. There was a past history of vasomotor rhinitis ascribed to an indefinite allergy of 15 years duration. Penicillin cream had been applied locally in a dispensary before admission resulting in an extension of the lesion with local itching. Treatment was begun, consisting of applications of Burow's solution to the lesion and 25,000 units penicillin intramuscularly every 3 hours. After the 3rd injection he developed an intense generalized disabling pruritus with a diffuse erythematovesicular rash over the neck and flexor surfaces of the arms with swelling and erythema of the inguinal folds. In the inguinal area, the vesicles reached the size of 1 cm. and exuded clear serum upon rupture. On Aug. 2, penicillin was stopped. He was now confined to bed, discomfort and malaise were intense, fever occurred daily from 99.5° to 100.5° F. The blood count showed an 8% eosinophilia with no other abnormal findings. On August 3, swelling of hands, fingers and toes with edema appeared. On mild local measures and symptomatic treatment all lesions subsided on August 7, when

he became afebrile and could be allowed out of bed. Desquamation in the antecubital spaces of both arms continued for several days. Repeat blood count was now normal, and there were no eosinophils in the differential smear.

was started on 25,000 units of penicillin intramuscularly at 3 hour intervals. Approximately 12 hours later, after the 4th injection, he developed a generalized erythema with pruritus and marked swelling of the hands and feet. Penicillin was stopped.

TABLE 1.—RESULTS OF VARIOUS TESTS WITH PENICILLIN IN 6 SENSITIVE PATIENTS

Case	Intracutaneous tests				Patch test		Conjunctival test (1:100)	Passive transfer test	Precipitin test
	1:10	1:100	1:1000	1:10,000	250 u./gm. emulsion base	Emulsion base only			
1*	+++	+++	++	++	++++	—	—	—	—
2*	+++	+++	++	—	+++	—	—	—	—
3*	—	—	—	—	+++	—	—	—	—
4*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5†	—	—	—	—	—	—	—	N.D.	—
6†	—	—	—	—	—	—	—	—	—
A‡	—	—	—	—	—	—	—	—	—
B‡	—	—	—	—	—	—	—	—	—
C‡	—	—	—	—	—	—	—	—	—
D‡	—	—	—	—	—	—	—	—	—
E‡	—	—	—	—	—	—	—	—	—

* Immediate reactions, due to drug allergy.

† Delayed reactions, simulating serum sickness.

‡ Controls.

TABLE 2.—PREADMINISTRATION TESTS FOR PENICILLIN SENSITIVITY IN 200 PATIENTS

	Penicillin	
	Brand 1	Brand 2
No. subjects tested	100	100
% positive:		
Intracutaneous tests:		
1:10 —		
1:100 —	100	5
1:1000—		
Patch tests, 250 u./gm. emulsion base	0	0
Conjunctival test, 1:100	0	0
Reactions, % positive following 50,000 u. penicillin after testing	0	0

Intracutaneous tests with penicillin solution in dilutions 1:10, 1:100, 1:1000, 1:10,000 were markedly positive after 3 hours and persisted for 72 hours. The patch test was markedly positive after 12 hours. Passive transfer, precipitin and conjunctival tests were negative.

CASE 2. A 34 year old ambulatory white male soldier entered the hospital on July 9, 1945, because of a severe spreading sycosis barbae of the face approximately 5 cm. in diameter. On July 16, the patient having experienced no improvement on other therapy, penicillin in emulsion base (250 units per gm.) was applied locally. Within 24 hours a marked local reaction of intense pruritus, redness and swelling occurred. Penicillin applications were discontinued and within 3 days all local manifestations of reaction had disappeared. On July 31 he

He was confined to bed. Blood count, previously normal, now showed a 13% eosinophilia. On August 4, following mild local treatment and symptomatic measures, all signs of reaction except slight itching had disappeared. His eosinophils were now 4%. Urine, Wassermann and stool examinations were normal. He gave no personal or family history of allergy.

Intracutaneous tests for penicillin sensitivity with dilutions up to 1:1000 were strongly positive after 90 minutes. A patch test with penicillin in emulsion base was likewise strongly positive after 30 hours. Passive transfer, precipitin and conjunctival tests were negative.

CASE 3. A 27 year old ambulatory white male soldier entered the hospital on Sept. 1, 1945, because of a severe warty dermatitis of the feet. He had urticaria

previous treatment with various remedies without success. Penicillin was started intramuscularly, 25,000 units every 3 hours. After 18 hours, following the 6th injection, he developed a severe pruritic erythematovesicular eruption over the entire body with marked swelling and edema of the face and genitals. The scrotum was covered with large vesicles which exuded clear serum. Previously ambulatory, he was now confined to bed. Discomfort was extreme, so penicillin was stopped. Mild soothing applications, epinephrine and ephedrine produced relief and on September 8 symptoms and reaction subsided. Blood count at the height of the reaction was normal except for a 32% eosinophilia which dropped to 23% within 1 week, then to normal on discharge. Stool, urine, serologic tests of the blood were normal. He gave no family or personal history of allergy.

The patch test was strongly positive within 24 hours and persisted until 96 hours. Intracutaneous tests performed with a brand of commercial penicillin other than the one which produced his reaction (no longer in stock) were negative in all dilutions. Passive transfer, precipitin and conjunctival tests were negative.

CASE 4. A 42 year old ambulatory white male soldier was admitted to the hospital on Mar. 16, 1945, for a chronic disabling bilaterally symmetrical dermatitis infectiosa eezematoides of both feet. Following ineffective treatment for 2 weeks, penicillin soaks, 250 units per cc. of normal saline solution, were applied locally. Within 24 hours he developed a chill with fever up to 101° F. and a generalized erythematous eruption over the entire body. Itching was intense. He was confined to bed and penicillin stopped. Within 48 hours on symptomatic treatment, the temperature returned to normal and all signs of reaction disappeared. On April 10, with no improvement in the original condition, it was decided to try parenteral penicillin; 25,000 units were given intramuscularly every 3 hours. Following the 3rd injection, he experienced another severe chill with fever to 102° F. and a recurrence of his former cutaneous reaction. With discontinuance of penicillin, the fever and skin manifestations disappeared within a few days. At this time, there was great need for hospital beds on Luzon and he was evacuated before further

investigative studies could be done. In spite of this, however, his reaction was so typical, its reproduction by readministration of the drug so definite, that this case warrants inclusion in this group.

CASE 5. A 26 year old ambulatory white male soldier had been under treatment in the dispensary for recurrent penicillin-resistant gonorrhea. He had received 3 courses of 100,000 units each on July 20, 24 and 27. On July 30 he was admitted to the hospital for a severe generalized urticaria gigantosa which had appeared the day before. Temperature was 101.6° F. which persisted for the next 6 days, during which he developed severe joint pains with swelling of both ankles and knees. He was dyspneic and the chest showed scattered wheezing râles which disappeared after repeated doses of epinephrine hypodermically. On August 8 the temperature subsided, on August 10 he was asymptomatic; on August 20 he was given 100,000 units penicillin in four 2 hourly doses. Immediately thereafter he again developed urticaria which required several doses of epinephrine in the course of the day to relieve. Temperature, blood count, urine, Wassermann test and stool examinations were normal. While he gave no personal history of allergy, a maternal grandmother was a sufferer of asthma.

Intracutaneous, patch, precipitin and conjunctival tests were negative. Passive transfer test was not performed because of danger of transmitting an undiagnosed syphilis.

CASE 6. A 20 year old colored male soldier was admitted to the hospital on July 21, 1945, acutely ill, toxic, suffering from an acute inflammatory process of the lower back muscles. His temperature spiked to 104° F. daily, leukocytes were 17,600 with 74% neutrophils. Following 3 days administration of penicillin, 200,000 units intramuscularly per day in divided doses, the temperature reached normal and symptoms subsided. No further treatment was given. Seven days following the last dose of penicillin, he suddenly developed a generalized severe giant urticaria, pruritus and fever to 101° F. Acute joint pains involving knees, wrists, ankles and hands appeared during the next few days with continuing fever. Repeated doses of epinephrine hypodermically and soothing local applications produced relief and he became

afebrile on August 7. On August 9 the urticaria disappeared and on August 11 he became symptom-free. Blood count, urine, stool and Wassermann examinations were normal. There was no personal or family history of allergy.

Discussion. The reactions in Cases 1 to 4 are examples of drug allergy caused by a drug allergen. According to the classification of Sulzberger and Goodman, cited by Sutton and Sutton,⁸ drug allergens are not antigens and they evoke no antibodies. Therefore, reactors fail to produce antibodies in their blood sera as denoted by negative passive transfer and precipitin testing. They do, however, react positively to skin test, and readministration of the drug will reproduce the reaction. The evidence and findings in Cases 1 to 4 satisfy these criteria and suggest that a drug allergen in commercial penicillin is the responsible agent for these reactions.

The composition of commercial penicillin is approximately 65% pure crystalline sodium penicillin and 35% impurities. The question arises therefore whether the allergen is contained in the penicillin or in the impurities. Silvers⁷ has reported a case of contact dermatitis due to a commercial brand of penicillin in which his patient failed to react when tested with pure crystalline sodium penicillin. He concluded, therefore, that the contact dermatitis was due not to the drug but to the impurities in the commercial product with which his patient had come in contact. On the other hand, Pyle and Rattner⁹ have reported a similar case of contact dermatitis to commercial penicillin which yielded a positive patch test to pure crystalline sodium penicillin indicating that the drug was responsible for the reaction in their case. Pure crystalline sodium penicillin was not available at this station, hence we were unable to differentiate in our cases whether the pure drug or the impurities were responsible for the reactions. However, in Case 3, the negative intradermal test was obtained from a brand of commercial

penicillin other than the one which had produced the reaction, suggesting that in this case at least the impurities accounted for the reaction. Criepp⁴ has reported a case of allergy to penicillin in which he was able to obtain positive skin, passive transfer and precipitin tests denoting the presence of reagins in the patient's serum. We were unable to duplicate this finding in our patients.

Cases 5 and 6 differ in that the reactions were delayed and simulated serum sickness. In such delayed reactions, negative skin and conjunctival tests are to be expected. As discussed by Cooke,³ little is understood regarding the mechanism of the production of delayed reactions. Case 5 resembled the case of severe asthma and skin reaction reported by Price, McNairy and White.⁵ Our patient likewise developed asthma and skin reaction but did not reach the critical stage bordering on exitus as did theirs. They suggest the sensitizing agent was not the active principle of penicillin but the impurities in the commercial make.

In an attempt to anticipate those individuals who might react untowardly to penicillin, 200 well patients awaiting discharge were tested on skin and conjunctiva with 2 different brands of commercial penicillin, 100 patients with each. Of these subjects, 50% had received penicillin at some time prior to this experiment and 50% had not. Following the reading of the tests each was given 50,000 units penicillin parenterally of the same make with which he was tested. The results are shown in Table 2.

While 200 cases are not sufficient for definite conclusions to be drawn in such an experiment, it will be noted that pre-administration testing for penicillin sensitivity in healthy subjects is of questionable value. The intracutaneous test proved inconsistent, the positives failed to react on test dose administered parenterally. Our experience with conjunctival testing in severe reactors (Table 1) throws doubt on this procedure in spite of the 200 negatives obtained (Table 2) in 200

non-reactors. No opinion is offered regarding the reliability of the patch test. Our experience with it in severe reactors (Table 1) and the work of Cohen and Pfaff,² who found 5 patients (0.9% their series) with positive pre-administration patch tests who later developed severe reactions on administration of the drug, suggests that this procedure may prove of value for detecting penicillin sensitivity. However, the 24 to 48 hour waiting period required to read the patch test could prove a costly delay for the patient if the drug was withheld pending result of the testing.

With but 6 instances of severe reactions in 5000 penicillin-treated patients (only 0.12%), the practical value to the clinician of determining pre-administration sensi-

tivity with tests of doubtful reliability is nearly *nil*. Instead, the drug should be administered promptly when needed, and the physician should bear in mind that there does exist an occasional patient who cannot tolerate penicillin and in whom it may be necessary to discontinue its use.

Summary. 1. Six cases of severe allergic reaction to penicillin are presented in which discontinuance of the drug was necessary.

2. In 4, the reactions were immediate and suggested that a drug allergen in commercial penicillin was the causative agent; in 2, the reactions were delayed and simulated serum sickness.

3. In our experience, pre-administration testing for penicillin sensitivity was found to be neither reliable nor practical.

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ISOLATION OF A GLUCOSE CONTAINING CEREBROSIDE FROM THE SPLEEN IN GAUCHER'S DISEASE

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THEORIES concerning the nature of Gaucher's disease have undergone considerable revision since its original designation as a "primitive epithelioma of the spleen."² Schlagenhauser¹⁰ first characterized the entity as a systemic disease of the lymph-hematopoietic apparatus, an opinion concurred in by most subsequent observers. Later, Marchand⁸ stated that the disease was due to the deposition of an unknown substance in reticulo-endothelial cells. This substance was first identified as kersin by Lieb^{6,7} who studied the properties of the cerebroside isolated from the spleen of a patient with Gaucher's disease. Other investigators have substantiated this finding.

Two views have been expressed concerning the pathogenesis of this entity: (1) that it is a general disturbance in lipid metabolism which expresses itself in an accumulation of cerebroside in the blood and organs and leads secondarily to the storage of kersin in reticulum cells; (2) the assumption that it is a dysfunction of the enzyme systems of the reticular cells themselves which leads to the increased synthesis and storage of kersin within the cell. The failure to demonstrate an increased blood concentration of cerebroside at any time in Gaucher's disease has lent support to the latter view. Most investigators have studied the fatty acid component of the kersin isolated from spleens in these cases but have assumed the presence of galactose without adequately identifying it. Recently, 4 investigators^{1,2,12} have reported that the carbohydrate component of the cerebroside isolated from the spleens

of patients with Gaucher's disease was glucose rather than galactose. There is, however, at least 1 report in which the carbohydrate was identified as galactose by the osazone.^{6,7} If the presence of galactose is to be accepted in some cases of Gaucher's disease it would appear possible that there are 2 types of the disease, 1 in which a metabolic disturbance leads to the deposition of normal galactose-containing kersin and a second in which the recently described glycolipid is deposited. Whether this assumption is correct can only be determined by chemical studies of additional cases of Gaucher's disease. The recent observation of a patient with Gaucher's disease whose spleen was removed at operation afforded us the opportunity to study the carbohydrate fraction of the cerebroside isolated from the organ.

Case Report. D. A. H. (Bon Secours Hospital, No. 6217), a white 12 year old school girl, was admitted to the hospital on Feb. 17, 1914, complaining of "sore throat, swollen glands in the neck, and fever of 2 weeks duration." The patient was said to have had a grippal attack 2 weeks prior to admission to the hospital. One week before admission, she had had a severe episode of epistaxis. Diarrhea had been present for several days and a tarry appearance of the stools was reported. Symptomatic relief added no further significant anamnestic details. The family history was not contributory. Three siblings were in good health and exhibited no evidence of lipid metabolic disturbance. The patient had had 4 previous admissions to the hospital at the ages of 11 months, 6 years and 11 years during her 9th year. Splenectomy had

first noted during the latter 2 admissions when the organ was found to be 3 finger-breadths below the costal margin. The admissions in 1940 were necessitated by frequent episodes of epistaxis extending over a period of 1½ years.

Positive findings on physical examination on present admission were as follows: pallor of skin and mucous membranes; evidence of acute tonsillitis; hepatomegaly with the edge felt 5 cm. below the costal margin; splenomegaly with the tip felt at the level of the umbilicus.

was somewhat thicker than normal and displayed a few scattered fibrous adhesions. Examination of the hilar structures revealed no gross lesions. On cross-section, the cut surface was seen to have a beefy-red appearance and the Malpighian bodies were obscured. Microscopically, the entire architecture was distorted. The Malpighian bodies were of normal size and structure and appeared as isolated islands of lymphoid tissue in a pale-staining pulp (Fig. 1). This pallor was due to the presence of masses of large acidophilic cells containing a single

TABLE 1.—CHEMICAL ANALYSIS OF SPLENIC TISSUE

Moisture + acetone soluble material	76.1%
Ether soluble material	4.0%
Cerebroside: alcohol soluble material (after ether extraction), % dry weight	2.5%
Melting point (theoretical 178°)	176°
Nitrogen (theoretical 1.73%)	1.8%
Carbohydrate: % cerebroside:	
Recovery after 10 minutes hydrolysis	15.1%
Recovery after 1 hour hydrolysis	13.9%
Loss by fermentation	100.0%
Mucic acid	Neg.
Osazone	Glucosazone
Melting point (theoretical 210-212°)	207-208°

Laboratory Studies: Blood picture on admission: hemoglobin 8.7 gm., red cells 2.56 million, white cells 3800 (neutrophils 22%, lymphocytes 78%). Reticulocyte count 0.6%. Platelet count 160,160 (Olef technique.) Coagulation time 5 minutes 45 seconds (Lee-White technique). Bleeding time 1 minute 15 seconds (Duke technique). Prothrombin time 25 seconds (Quick technique). Urinalyses essentially negative. Blood chemistry: blood sugar 100 mg. %, non-protein nitrogen 30 mg. %; icterus index 5 units. Liver function tests: 4+ cephalin-cholesterol flocculation; intravenous hippuric acid test 0.68 gm. excreted in 1 hour. Bone marrow studies revealed an essentially normal myelogram.

Roentgen ray examinations of the femora were reported as normal. Roentgen examination of the esophagus with particular reference to varicosities revealed no such abnormality.

A preoperative impression of Banti syndrome was recorded and splenectomy advised. This was performed on Feb. 25, 1944, by Dr. Frank C. Marino. The surgical specimen consisted of the spleen, which weighed 822 gm., and a small biopsy fragment of liver. The capsule of the spleen

eccentric vesicular nucleus. The cytoplasm of these cells was seen to have a fibrillar structure. These cells fulfilled the cytological descriptions of so-called Gaucher cells. The liver biopsy displayed parenchymal cells which were swollen and vacuolated. No increase in interstitial fibrous tissue was seen.

Chemical studies of the spleen were carried out in the following manner: A portion of the spleen which had been preserved in formalin for 2 days was dried with acetone, powdered and extracted repeatedly with cold ether. It was then extracted with hot alcohol until no further cerebroside was obtained. The combined extracts were filtered and allowed to stand overnight at 5° C. The precipitate was centrifuged out and crystallized from hot alcohol. It was then dissolved in pyridine and precipitated with acetone. Following a crystallization from alcohol, the melting point of the nearly white lipid material agreed with that of kersin. A nitrogen content as determined by analysis by the microkjeldahl technique also agreed with that of this cerebroside. Since only the carbohydrate portion was to be studied, portions of the material were hydrolyzed by the short procedure of Kirk.⁴ The

hydrolysate was neutralized with sodium hydroxide and the reducing sugar determined before and after treatment with washed yeast for 1 hour at 37° C. The reducing substance was 100% fermentable. The percentage of reducing sugar before fermentation was somewhat low but little more was destroyed by 1 hour heating with

A summary of the data which lead to the conclusion that the carbohydrate component of the cerebroside isolated from this spleen is glucose follows:

The postoperative course of the patient was entirely uneventful. The blood picture showed improvement and the cepha-

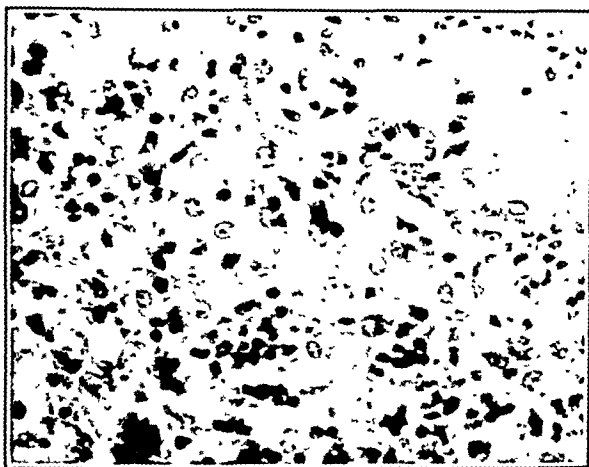


FIG. 1.—Detail of splenic pulp. Note presence of masses of large, pale-staining Gaucher cells. (X 400.)



FIG. 2.—Glucosazone obtained from the carbohydrate component of the cerebroside

the acid. The mucic acid test was negative. The α -azone, prepared by usual methods, and recrystallized from 70% alcohol, had the typical appearance of glucosazone. The melting point of the α -azone was 207° to 208°. This is slightly lower than that of pure glucosazone but definitely higher than that of galactosazone.

lin-cholesterol flocculation test returned to normal. On March 10, 1914, the patient was discharged in good condition.

Summary. 1. A case of Gaucher's disease in which the operatively removed spleen was subjected to chemical and pathologic study is reported.

2. The carbohydrate fraction of the lipid isolated from the spleen was found to be glucose. disease: one in which the lipid is a galacto-lipid similar to normal kersin, the other, an entity characterized by the

3. It is suggested that there may be 2 forms of lipid disturbance in Gaucher's accumulation of an abnormal glucose-containing kersin.

We wish to thank Dr. Frank J. Geraghty for permission to use the clinical data of the case reported.

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COMBINED QUININE-PLASMOCHIN TREATMENT OF VIVAX MALARIA: EFFECT ON RELAPSE RATE

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A STRIKING feature of vivax malaria of Pacific origin is its tendency to relapse after therapeutic termination of the acute attack with any of the antimalarial drugs currently in use. Of 583 patients treated by us for acute attacks and observed for a minimum of 120 days after treatment, 425 patients (73%) had a clinical relapse, *i. e.*, a recurrent attack of malaria with fever and blood smears positive for *P. vivax*. An additional 29 patients (5%) who did not have a clinical relapse had positive smears without fever or symptoms at some time while under observation after an attack. In other words, treatment of the attack failed to cure 78% of the patients; 259 patients who relapsed were again observed for 120 days after retreatment. Of these, 181 (71%) relapsed, and 15 (6%) who did not relapse clinically, had at some time during their period of observation after the acute attack, a positive smear without fever or symptoms. Thus, retreatment of the next attack again resulted in curative failure in 77% of the patients. Even if this trend of such a high failure rate continued after treatment of each successive attack, within 2 years after the first attack less than 2% of any large group originally treated would remain who might still have future relapses. However, the total number of attacks experienced within the group during the period of activity of the disease would be considerable. Present treatment is, therefore, not effective

in curing vivax malaria of Pacific origin in the majority of patients and is useful only in terminating episodes of acute clinical activity until the disease in any given patient has spent itself.

The purpose of this paper is to present the results of the combined use of quinine and plasmochin in the treatment of acute attacks of vivax malaria with particular reference to the effect of such treatment on subsequent relapse.

Background. Plasmochin was introduced as a new synthetic antimalarial drug by Schulemann and his co-workers at Leverkusen in 1924. While this drug has definite antimalarial activity and is effective in terminating the acute attack with relatively large doses, it was soon apparent that plasmochin by itself is not a practical drug in the treatment of acute attacks of malaria principally because of its potential toxicity. Acute attacks of malaria are more effectively and safely terminated by the use of quinine, quinacrine or more recently introduced 4-amino-quinoline compounds. In recent years plasmochin has been used almost entirely as an adjunct to other antimalarial therapy because of its ability to eradicate gametocytes of *P. falciparum* with small amounts of drug in a matter of a few days. The value of this practice as a control measure in endemic areas is questionable. However, there is evidence that the simultaneous administration of plasmochin and quinine daily for 2 weeks or more is highly

effective in reducing relapse rates in vivax malaria during an observation period of 2 to 6 months. This is in sharp contrast to curative failure or high relapse rates for Pacific vivax malaria treated with quinine, quinacrine, or 4-amino-quinoline compounds. It is not our purpose to review the voluminous literature on plasmochin but brief reference will be made to studies with plasmochin in relation to its effect on subsequent relapse and its toxicity.

Sinton and Bird⁹ in 1928 reported a study from India in which 86 patients who received plasmochin alone or plasmochin and quinine together for an attack of vivax malaria were followed for relapse for 2 to 4 months after treatment. Twenty-nine patients received 0.08 gm. of plasmochin (probably plasmochin naphthoate) on 17 treatment days during a total of 39 days as suggested by the German manufacturers of the drug. The relapse rate during the period of observation was 36%. Twenty-two patients received 0.08 gm. on as many consecutive days as possible for 28 treatment days with interruptions only for toxic manifestations. The average period during which the 28 treatment days were given was 36, with a range from 28 to 53 days. The relapse rate in this group was 23%. Fifteen patients received 0.1 gm. plasmochin plus 1.25 gm. quinine sulphate on 17 treatment days during a 39 day course of treatment. The relapse rate in 2 to 4 months was 20%. Finally a group of 20 patients received these same daily amounts of both drugs for 28 days as continuously as possible during an average treatment period of 37 days. No patient relapsed during the period of observation. The relapse rate for the 51 patients who received plasmochin alone was 30% and 8.5% for the 35 patients who received plasmochin and quinine together. Thus, of 86 patients in all 4 groups who received plasmochin for 17 to 28 days, relapse rate as given by the authors was 21%. Six patients who were lost sight of or who did not complete the

full course of treatment were counted as failures so that the failure rate actually observed for 80 men during a period of 2 to 4 months after treatment was 16%. In contrast to this finding the relapse rate for 111 men treated with quinine alone and similarly observed was 77%. There is little question from the results of this study that combined quinine-plasmochin treatment very substantially reduced the incidence of relapse during 2 to 4 months after treatment.

Sinton *et al.*¹⁰ in 1930 reported relapse rates for 2 additional groups of patients who received combined quinine-plasmochin treatment for acute attacks of vivax malaria. Seventeen patients received plasmochin, 0.06 gm., and quinine sulphate, 1.25 gm., daily for from 4 to 21 days as follows: 4 patients 4 days, 7 patients 21 days, and 1 patient each for 8, 9, 11, 14, 16 and 17 days, respectively. No patient in this group had a clinical or parasitemic relapse for 2 months after treatment when the experiment was terminated. An additional 44 patients received plasmochin, 0.04 gm., and quinine, 1.25 gm., daily for 21 days. Three patients relapsed making a total failure rate of 6% for 54 men who received plasmochin for 14 or more days in contrast to a relapse rate of 42% for 38 patients who received quinine alone.

Jarvis⁶ in 1932 reported 8% relapses during a 2 to 4 months period of observation for 75 patients who received plasmochin, 0.03 gm., and quinine, 1.3 gm., daily for 21 days. Manifold⁷ in the same year reported a large scale study of some 3000 Indian and British troops who were treated for acute attacks of vivax malaria with plasmochin, 0.04 gm., and quinine, 1.3 gm., daily for 21 consecutive days. Of these, 98% were able to complete the full course of treatment. Records of readmission for relapse were analyzed during a period of 5 months after treatment and the relapse rate for the whole group was 5.2% in contrast to a wide experience with quinine alone in which relapse rates were from 42 to 77% after treatment.

More recently, Thomson and William¹² (1945) reported an extensive study on the effect of combined quinine-plasmochin treatment of vivax malaria of Mediterranean origin in this war. Of 100 patients with delayed primary attacks of vivax malaria treated with 4.6 gm. of quinacrine during 12 days, 29% relapsed during an observation period of 5 months. Of 76 patients with delayed primary attacks of vivax malaria treated for 10 days with plasmochin base, 0.03 gm., and quinine, 2 gm., daily, only 14 (18%) relapsed. The relapse rates for 650 men treated with quinacrine as above for later relapses and followed for 5 months was 34%, while the relapse rate for 584 men treated for later attacks with combined quinine-plasmochin was 10.3%. In our experience the relapse rate in 120 days for Mediterranean vivax malaria (150 patients) treated with quinacrine or quinine was 32%. There can be no question about the significance of the reduced relapse rate in this British report.

Thus far, references have been cited which indicate that combined quinine-plasmochin given for at least 10 days during which amounts of plasmochin base are at least 0.03 gm. daily is highly effective in reducing the relapse incidence of vivax malaria during an observation period of 2 to 5 months. Experiences with smaller amounts of plasmochin or short term schedules are generally not convincing and definitely of no value in Pacific vivax malaria. Dieuaide,³ in reviewing relapses following various schedules of treatment of vivax malaria in the Pacific, cites a relapse figure of 83% within 16 weeks for 185 men who received 2 courses of quinacrine hydrochloride for acute attacks of vivax malaria, and a relapse rate of 78% for 136 patients who received 2 consecutive courses of treatment consisting of 0.1 gm. quinacrine 3 times daily for 7 days followed by plasmochin naphthoate, 0.02 gm., 3 times daily for 5 days, after which both were repeated. Thus, plasmochin for 5 days and repeated a week later had no effect in reducing the relapse

rate compared with quinine or various schedules of quinacrine mentioned by Dieuaide. In the *Bulletin* of the U. S. A. Med. Dept.,² relapse rates are compared for various groups of patients treated in this country for acute attacks of vivax malaria of Pacific origin with and without plasmochin and followed for at least 90 days afterward. Of 176 patients who received quinacrine or totaquine alone, 57% relapsed. To 299 patients was given 0.01 gm. plasmochin base 3 times daily for 3 days after quinacrine or totaquine alone, or after quinine or totaquine and quinacrine. The combined relapse rate in all these plasmochin groups was 56%. Here again is good evidence that plasmochin for 3 days after other antimalarial therapy is not effective in influencing the relapse rate of Pacific vivax malaria. On the other hand, Gentzkow and Callender¹ in 1938 reported from Panama that this amount of plasmochin (0.01 gm. t.i.d. for 3 days) in addition to 2.4 gm. of quinacrine during 4 days given to 128 patients resulted in a relapse rate of 9.4% in 6 months, compared with 45.6% relapse for 215 patients who received quinacrine alone. This report is based on analysis of patient malarial registers. From India, Bird¹ reported 30.9% relapses in 152 patients treated with plasmochin (0.01 gm. t.i.d. for 5 days) after 5 to 7 days of quinacrine, and a relapse rate of 46.3% for 201 patients who received the same amount of plasmochin after 7 days of quinine. These observations suggest that plasmochin for 5 days after quinine or quinacrine is not effective in reducing relapse rates in India compared with simultaneous quinine and plasmochin treatment for 10 to 14 days.

Summarizing the work cited, the evidence indicates definitely that simultaneous quinine-plasmochin treatment for 14 days or more of vivax malaria of Indian or Mediterranean origin results in a very significant reduction in relapse rates during periods of observation of from 2 to 6 months, and that plasmochin for 3 or 5 days after quinine, totaquine, or atabrine

is of no benefit in reducing relapses in vivax malaria of Pacific origin. With regard to the period of observation referred to above for these studies, it should be borne in mind that the majority of relapses occur within a month after quinine or totaquine treatment and within 3 months after quinacrine or 4-amino-quinoline drugs. Short-term courses of plasmochin (3 to 5 days) are of doubtful value in reducing relapse rates in vivax malaria of Indian origin and the reported beneficial effect of 3 days of plasmochin after quinacrine in reducing relapses in vivax malaria of Panamanian origin remains unconfirmed.

The present report deals with the effect on subsequent relapse of simultaneous combined quinine-plasmochin treatment for 14 days in vivax malaria of Pacific origin, an aspect that has not heretofore been studied.

Materials and Methods. The protocol herein described was furnished by the Office of the Surgeon-General. Seventy-two white patients with acute attacks of vivax malaria of Pacific origin having fever and positive smears were admitted to a special treatment and study ward. No attempt at selection of patients were made. All drugs were administered by an officer. On the 1st day, quinine sulphate, 1 gm., and plasmochin naphthoate, 0.02 gm. (0.01 gm. base), were given together at 8 hour intervals. On Days 2 to 14 inclusive, quinine sulphate, 0.65 gm., and plasmochin naphthoate, 0.02 gm., were given together at 8 hour intervals. The total amount of drugs administered during the treatment period of 14 days was 29 gm. of quinine sulphate and 0.84 gm. of plasmochin naphthoate. A control group of 75 white patients with acute attacks of vivax malaria of Pacific origin were treated with 29 gm. of quinine alone during 14 days on the above schedule. Temperatures were recorded every 4 hours. Parasite counts were done twice daily until negative for 3 consecutive days. Hemoglobin, methemoglobin, and total white blood count determinations were made daily. (Hemoglobin and methemoglobin were determined colorimetrically as cyan-methemoglobin.) Each patient was examined at least once daily

by a medical officer and special attention was paid to the recognition of possible manifestations of plasmochin toxicity. On the day after completion of treatment on the ward, the patients were transferred to a convalescent area for further observation. The duration of observation was until relapse or for a minimum of 120 days. During this interval smears were examined twice weekly. In the event of parasitemia, temperature observations were made every 4 hours and parasite counts were done daily. A temperature of 100° F. or more, by mouth, associated with a positive smear was considered a clinical relapse.

Results. The cumulative clinical relapse rates during 120 days observation after treatment of acute attacks with quinine, quinacrine and combined quinine-plasmochin are shown graphically in Chart 1. The total failures after treatment are summarized in Table 1.

A. QUININE. Seventy-five patients with acute attacks of vivax malaria of Pacific origin were treated with 29 gm. of quinine sulphate during 14 days. Sixty-two patients (85%) had a clinical relapse within 120 days. Five patients who did not relapse had, at one time or another during their period of observation, a positive smear without fever or symptoms. Thus quinine failed to eradicate the infection in 67 (89.4%) of patients treated and observed for 120 days.

B. QUINACRINE (ATABRINE). Sixty-nine patients with acute attacks of vivax malaria of Pacific origin were treated with 2.8 gm. of quinacrine during 7 days. Fifty-six patients (81.1%) had clinical relapses within 120 days after completion of treatment. An additional 2 patients who did not relapse had, at one time or another during their period of observation, positive smears without fever or symptoms. Thus, within 120 days following quinacrine, 84% of treated patients exhibited evidence that they were not cured of the infection.

C. Combined Quinine Plasmochin. Seventy-two white patients with acute attacks of vivax malaria of Pacific origin were

treated with plasmochin and quinine as described under "Materials and Methods" above. Three clinical relapses occurred within 120 days after completion of treatment or a clinical relapse rate of 4%. Five additional patients who had

Toxicity. Numerous references can be found in the literature dealing with toxic manifestations of plasmochin of varying type and severity. The principal toxic symptoms or signs reported are gastrointestinal complaints: mild to severe epi-

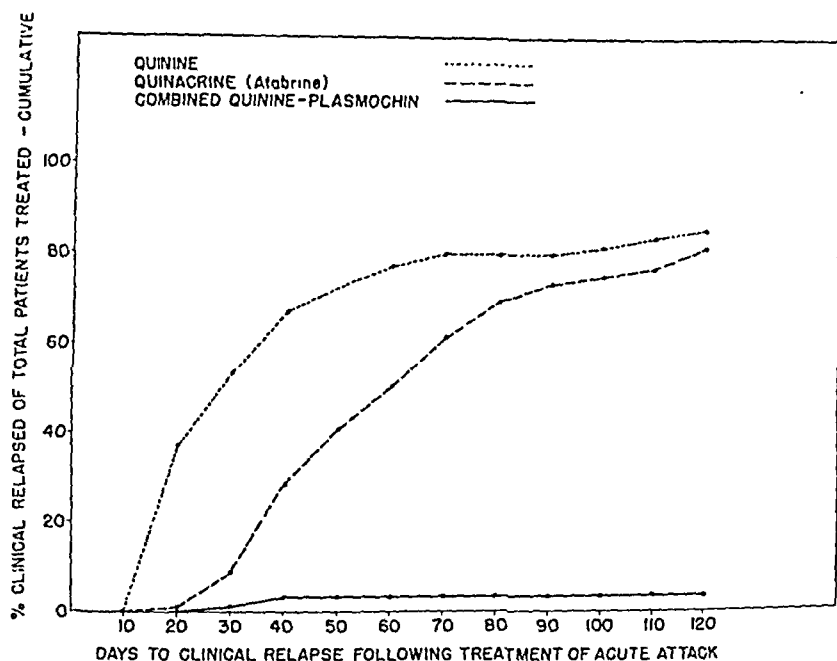


CHART 1.—Comparative clinical relapse rates (cumulative) after treatment with quinine, quinacrine or combined quinine-plasmochin.

Three groups of patients treated after acute attacks of vivax malaria of Pacific origin. (See text for dosages.) These patients were all followed for a minimum of 120 days after treatment of the acute attack.

TABLE 1.—RESULTS OF TREATMENT IN 3 GROUPS OF PATIENTS WHO RECEIVED QUININE, QUINACRINE, OR COMBINED QUININE-PLASMOCHIN FOR AN ACUTE ATTACK OF VIVAX MALARIA OF PACIFIC ORIGIN AND WERE THEN FOLLOWED EITHER TO RELAPSE OR FOR 120 DAYS AFTER COMPLETION OF TREATMENT (FOR DOSAGES, SEE TEXT)

	Patients treated	Previous relapses (av.)	Prior activity (av.)	Relapses					
				Clinical*		Parasitemia only†		Total failure-‡	
				No.	%	No.	×	No.	%
Quinine	75	3.8	9.1	62	82.8	5	6.7	67	89.3
Quinacrine	69	4.4	7.8	56	81.1	2	2.9	58	84.0
Quinine and plasmochin .	72	5.2	10.6	3	4.2	5	6.9	8	11.1

* Clinical relapse—clinical recurrence with fever, symptoms and positive blood smear, observed within 120 days after treated attack.

† Parasitemic relapse—positive smear only without fever or symptoms and not followed by clinical relapse during 120 days observation after treated attack.

‡ Total failures—clinical relapses plus parasitemic relapses during 120 days after treated attack.

neither fever nor symptoms during the 120 days observation showed parasitemia in this period. Thus, of 72 Pacific infections treated there were altogether only 8 failures making a total failure rate of 11.1% for the 120 days.

gastric pain or soreness, anorexia, abdominal cramps, nausea, vomiting and diarrhea; cyanosis; circulatory system signs and symptoms consisting of dyspnea, changes in pulse, blood pressure and electrocardiograms; changes in the blood

varying from mild anemia to severe or fatal hemolytic crises or agranulocytosis; vague muscular aches and pains and weakness; and central nervous system symptoms principally headache, dizziness, "nervousness," psychosis and coma. The incidence of severe toxic reactions from the drug varies from 1 to 10% in different series reported. The hemolytic reaction is the most serious manifestation of plasmochin intoxication and may vary from mild progressive anemia to a sudden severe and fatal hemolytic crisis associated with shock, severe anemia, jaundice, hemoglobinuria and azotemia. This reaction may come on early after relatively small amounts of drug but also may occur at any time during the administration of plasmochin. Weakness and dark urine are the most common symptoms at onset. During the acute episode the erythrocyte sedimentation rate, the white blood count, the reticulocytes, and serum bilirubin are elevated, while the red blood count and hemoglobin fall. Race, diet, climate and the prior administration of other drugs have all been suggested as factors which may be responsible for initiating a hemolytic reaction. Evidence regarding the possible relation of race and predisposition to hemolytic reactions will be presented. The degree of methemoglobinemia in many individuals is probably related to the dose of drug. Gastro-intestinal symptoms are most common during the 4th and 5th days of therapy, and Roentgen ray studies during this time have revealed gastric hyperperistalsis and intestinal spasm. Brief reference will be made to the incidence of toxic experiences in relatively large groups recently reported.

Manifold⁷ (1931), in a report dealing with the results of treatment of vivax malaria in India with quinine, 1.25 gm., and plasmochin, 0.01 gm., given together daily for 21 days, presents an analysis of toxicity in 3213 patients so treated. Toxic symptoms or signs occurred in 21% of 1298 British soldiers and 10% in 1915 Indians treated. Epigastric pain or other

gastro-intestinal symptoms were noted in 15% of the British and 8% of the Indian patients. Cyanosis was observed in 4% of the British cases, but this was not accurately determined in the Indians because of their color. Jaundice was observed in only 3 patients and in 1 of these, an Indian, death followed as a result of a severe hemolytic reaction. In the author's opinion, the majority of the symptoms were mild and he emphasizes that of 480 British patients personally treated the full course of 21 days of plasmochin was completed without interruption by 478. He also states that 98 to 99% of the patients in the whole series of more than 3000 cases completed a full course of treatment.

West and Henderson¹³ (1944) reported an incidence of 2.85% plasmochin toxicity in 846 patients treated for *P. falciparum* infections in Africa with quinine, 2.0 gm. daily for 3 days, quinacrine, 0.3 gm. daily for 5 days, no treatment for 2 days, and plasmochin base, 0.03 gm. daily for 5 days. Twenty-two of the 24 patients with toxic signs had primary Falciparum infections and the other 2 had had malaria and plasmochin previously. Four patients were hospitalized after 0.06 gm. of plasmochin base and the mean total toxic dose was 0.119 gm. Jaundice was the most common finding and was present in 20 patients, the mean icterus index being 27.6. Abdominal pain was present in 20 patients. Headache, weakness and dizziness were present in 16, 14 and 11 patients, respectively. Two patients were psychotic and 1 in coma. Anemia was present in 19 patients and in 1 the red count was 1.4 million per c.mm., the average being 2.88 million per c.mm. The white counts varied from 4.5 to 20.8 and averaged 10.2 thousand per c.mm. All patients except 1 were ambulatory during the period of plasmochin therapy. Unfortunately, the color of the patients or the ratio of black to white in the population sample reported is not stated in this paper. This is of particular interest, because Swantz and Bayliss¹¹ in 1945

reported moderately severe hemolytic toxic reactions in 9 Negroes who had received plasmochin in the course of treatment for malaria. These reactions were encountered during a period of time in which approximately 3000 cases of malaria were treated. The amount or duration of plasmochin treatment is not stated and while exact racial data are not presented the authors state that the majority of their malaria cases were white. The absence of a single hemolytic reaction in the whites treated and the occurrence of 9 cases of severe intoxication in Negroes may suggest some predisposition to plasmochin hemolytic reactions in Negroes. Further evidence of this nature is suggested by Shannon *et al*,⁸ who observed 6 hemolytic reactions (5 Negroes and 1 Chinese) among 71 pigmented patients and none in 35 white patients who received 0.03 gm. plasmochin base for from 2 to 14 days. The incidence of hemolytic reactions for the pigmented group was 8.4%. All reactions occurred during the 3rd to 5th days of plasmochin therapy and were characterized by weakness, dark urine and icterus at onset.

Hardgrove and Applebaum⁵ (1945) reported from Panama an incidence of 10.13% of plasmochin poisoning in 4361 laborers who received a routine mass treatment for malaria suppression consisting of quinacrine, 0.1 gm., 3 times daily for 5 consecutive days, no medication for the next 2 days, and then plasmochin base, 0.01 gm., 3 times daily for 5 consecutive days. The reactions were of sufficient severity to require hospitalization of 8% of the treated group. It is of special interest that the incidence of hemolytic reactions in the whites treated with plasmochin was 5.8%, and 11% in the other consisting principally of Negroes. Only 21 toxic reactions occurred following less than 0.1 gm. of plasmochin base. Three-fourths of the admissions took place during the first 3 days of plasmochin treatment. The principal symptoms were abdominal pain, dark urine, anorexia, jaundice, headache, nausea and vomiting.

The important clinical findings were jaundice, abdominal tenderness, enlarged liver, pallor, cyanosis, low-grade fever, hemoglobinuria, bilirubinemia, anemia and leukocytosis. No deaths occurred, and treatment was essentially blood transfusion, intravenous glucose solution and sodium bicarbonate by mouth. It should be noted in this series that the incidence of hemolytic reactions was twice as great in whites as compared with Negroes and others, and that plasmochin was administered to men while they were working.

Thomson and William¹² in their report dealing with 660 British soldiers who were treated for acute attacks of vivax malaria with quinine, 2 gm., and plasmochin base, 0.03 gm., daily for 10 consecutive days, encountered practically no severe toxic manifestations. Of 295 patients personally treated by the authors, in only 3 was therapy interrupted because of toxicity. Other medical officers participating in the study, having less experience with plasmochin and being more cautious, interrupted therapy in 2 to 4% of 365 subjects. No serious reactions were encountered.

It is evident from a brief survey of the literature that toxic experiences with plasmochin vary considerably in their incidence and severity. Large groups of patients have been given 0.03 gm. of plasmochin base for 10 to 21 days with practically no toxicity. On the other hand, serious hemolytic reactions have been reported when the drug was given for only 3 to 5 days. It is probable that Negroes are more susceptible to hemolytic reactions than whites and that taking plasmochin under working conditions may be a factor in producing serious reactions.

We have administered plasmochin naphthoate in doses of 0.02 gm. 3 times daily at 8 hour intervals for 14 days to 100 white patients. No major toxic manifestations were observed and all patients were able to complete the full course of therapy.

Forty % of the patients had some form of complaint referred to the gastro-intestinal tract and probably related to plas-

mochin. These complaints consisted principally of abdominal cramps or abdominal soreness which were rarely severe. These gastro-intestinal symptoms usually began from the 3rd to the 5th day and lasted from 1 to 7 days.

Cyanosis was observed in 1 patient who had a methemoglobin value of 12% on the 11th day of treatment. Ninety % of the patients showed methemoglobinemia above normal values at some time during treatment with amounts ranging from 1 to 12% (average of 2.3%) of total hemoglobin.

In 16% of the patients who received plasmochin for 14 days there was a fall in total hemoglobin of from 11 to 20% which occurred during the 2nd week of treatment which we considered related to plasmochin. An equal number of patients had an average fall in hemoglobin of 15.3% during the first 5 days. In the latter group, this fall in hemoglobin was apparently due to active malaria rather than plasmochin, since it occurred in the first few days of the acute attack and reversed itself with continued treatment. Severe anemia did not occur and no hemolytic crisis was observed.

The effect of plasmochin on the white blood count was to produce leukocytosis in a significant number of men (15% of counts above 10,000 per c.mm.) during the 2nd week of treatment and leukopenia (24% of counts below 5000 per c.mm.) during the 1st week after discontinuance of the drug. Subsequent counts 2 weeks after treatment were all normal.

In our experience the toxic manifestations related to the administration of plasmochin were not severe or serious and should not detract from the value of the drug if proper care is taken in recognizing potentially serious signs of toxicity.

Evidence has been presented that hemolytic reactions may occur more frequently in patients who are not white and some investigators believe that toxic manifestations due to plasmochin are more common in patients who have recently received quinacrine. Plasmochin plasma levels are very much higher in patients

who have recently received quinacrine in comparison with levels from patients who have not had the drug. But whether high plasmochin levels and toxicity are related has not been established. It is suggested that patients receiving plasmochin as described in this paper be hospitalized during treatment and frequently observed to recognize early severe hemolysis, should it occur. Treatment should be limited to white patients. Hemoglobin determinations should be performed daily and complete blood counts at least twice a week. Cyanosis alone is not an indication for discontinuance of therapy. A fall in total hemoglobin of more than 20% in any one day should be regarded with suspicion and, if followed by a further decline in the amount of total hemoglobin on the next day, plasmochin treatment should stop. One cannot anticipate sudden hemolysis by any laboratory method but symptoms of severe weakness and dark urine during the first 5 days of therapy should be investigated from the standpoint of a beginning hemolytic reaction. Fluids, blood and alkalies by vein are indicated if a severe reaction should occur. Abdominal cramps occur most frequently during the first week of treatment and if severe may be controlled with atropine.

The usual symptoms of cinchonism, namely, tinnitus, fullness in the head and ears, and headache, were encountered in varying severity in the 1st week of treatment in the majority of patients. These symptoms gradually subsided in the 2nd week and caused no interruption of therapy.

During the first 2 to 5 days of the acute attack of malaria most patients will remain in bed. For the remainder of the 2 week period of treatment in the hospital the patients may be ambulant on the ward, but vigorous exercise or overnight passes should not be allowed. Each dose of drug must be personally administered by a nurse or physician and each patient should be seen at least twice daily. Patients with anemia, severely malnourished, or in poor

physical condition should not be treated with plasmochin.

Discussion. The clinical relapse rate of only 4% and a total failure rate of 11.1% following combined treatment with quinine and plasmochin is very striking. In our experience, the clinical relapse for 10 groups of at least 50 patients each has varied from 65 to 85%, with total failure rates after treatment of from 75 to 90% for all groups except the quinine-plasmochin group herein reported.

Analysis of more than 1000 attacks of vivax malaria treated and observed here for 120 days indicates that within this period of observation the relapse rate after any given attack is not significantly influenced by the number of previous attacks, age of the disease, or amount, or duration of treatment with quinine or quinacrine. It is unlikely that these factors (see Table 1 for comparison of age of disease and number of previous attacks) can account for the great difference observed between the failure rates of 11.1% following combined quinine-plasmochin treatment and 85 to 90% observed after quinacrine, quinine or other antimalarial drugs we have studied. The significance of the 120 day period of observation after treatment should be emphasized at this point in relation to the probability of late relapses occurring after 120 days in the quinine-plasmochin group. The median interval to clinical relapse following treatment with quinine or totaquine is 24 days and 50 to 65 days following quinacrine or 4-amino-quinoline drugs. Within 120 days 80 to 90% of all patients treated for an acute attack of vivax malaria of Pacific origin with currently used antimalarial drugs will have interval parasitemia without fever or symptoms or actually relapse clinically. We know that a relatively small percentage of failures do occur after 120 days. It is unlikely that absolutely no definitive cures follow treatment with quinine or quinacrine but even if this were so, the maximum number of failures which could possibly occur after 120 days observation would be only 10 to 20% of

treated patients. The median interval to failure (parasitemic and clinical) observed in the relapses which actually occurred in the quinine-plasmochin group was 34 days, compared with 36 days for the quinine controls. In other words, when quinine-plasmochin failed, it did so in the same interval after treatment as following quinine alone. We have now observed 20% of the plasmochin group for at least 180 days after treatment and no failures after 120 days have occurred. Unless plasmochin alters the biology of vivax malaria in man so that very late failures will occur in the majority of treated patients, it is our opinion that freedom from parasitemia or clinical relapse for 120 days which occurred in 90% of our treated patients represents definitive cure for at least 80% of men so treated.

Analysis of our plasmochin relapses gives us no clue to a possible explanation for failure. The average mean quinine and plasmochin plasma levels in the failures are in the same order and range as observed for the whole group treated, as well as for the patient in whom 120 day cures were observed. There was 1 failure of 9 delayed primary attacks treated. The average age of the disease and the average number of previous attacks in the other 7 failures was comparable to the patients in whom treatment was successful.

Four month cures in 90% of patients treated with combined quinine-plasmochin raises the question of the general application of this form of treatment for vivax malaria. The relatively low clinical relapse rate of 30% within 120 days for vivax malaria of Mediterranean origin following treatment with quinacrine or quinine makes it questionable whether plasmochin treatment is routinely indicated in such infections, especially in the 2nd year of the disease. Certain individuals with vivax malaria of Mediterranean origin relapse frequently and at short intervals after treatment during the 1st year of the disease, particularly if quinine is used to terminate the acute attack. In these cases, plasmochin-quinine should

be considered, even though the likelihood of relapse for large groups of Mediterranean vivax cases is only about 30% in 120 days following other forms of therapy.

Since 120 day failure rates after treatment of attacks of Pacific vivax malaria may be as high as 90%, more serious consideration should be given to the use of combined quinine-plasmochin in this type of infection. Even though there is only a 10 to 20% chance that a patient treated with quinine or quinacrine for his first attack will have no subsequent attack in 120 days, it seems worthwhile to take this chance for that attack and possibly for the next one or two relapses. However, the occurrence of repeated attacks at short intervals, for example, 3 to 6 attacks during the first 6 months of the disease or repeated attacks later in the disease, is in our opinion an indication for the use of combined quinine-plasmochin therapy. The coexistence of other diseases which preclude the use of quinacrine therapeutically or for suppression (quinacrine sensitivity and/or exfoliative or eczematoid dermatitis or atypical lichen planus) is another indication for the use of combined quinine-plasmochin. Likewise, patients who relapse frequently at intervals of a month or less after quinine and who cannot take quinacrine for the reasons mentioned above can be treated with plasmochin as outlined in this paper. Finally, patients whose convalescence from other diseases is interrupted or delayed by repeated attacks of malaria should be considered candidates for combined quinine-plasmochin therapy. Each case must be considered individually and the merits of possible cure weighed against potential toxicity and a 2 weeks course of hospital treatment with plasmochin compared to a short safe course of treatment with other antimalarial drugs, although failure from the latter is to be expected in a very high percentage of treated patients.

We appreciate the potential dangers of plasmochin, but we are impressed by the complete absence of severe or serious toxicity in our series of 100 consecutive

white patients to whom we have given 0.06 gm. plasmochin naphthoate daily for 14 days. The fact that our patients were all white, closely observed in the hospital, and in good physical condition may be a factor in the absence of toxicity.

Summary and Conclusions. 1. Quinine sulphate, 1 gm., and plasmochin naphthoate, 0.02 gm., simultaneously at 8 hour intervals for 1 day followed by quinine, 0.65 gm., and plasmochin naphthoate, 0.02 gm., simultaneously at 8 hour intervals for the next 13 consecutive days were administered to 72 white patients with acute attacks of vivax malaria of Pacific origin who were followed for at least 120 days after treatment.

2. The clinical relapse rate and total failure rate during 120 days observation was 4 and 11.1% respectively, following the above course of treatment. This is in sharp contrast to clinical relapse rates of 75 to 85% and total failure of 85 to 90% based on similar observations after treatment of more than 500 patients for acute attacks of vivax malaria of Pacific origin with quinine, quinacrine or other antimalarial drugs.

3. Combined quinine-plasmochin treatment resulted in apparent cure in 90% of patients as judged by the occurrence of clinical relapse or appearance of parasites without fever or symptoms during a period of 120 days after treatment. According to the same criteria, only 10 to 15% of cures follow treatment with quinine, quinacrine or other antimalarial drugs currently in use.

4. No conspicuous or serious toxic manifestations were observed in 100 white patients who received combined quinine-plasmochin therapy for 14 consecutive days. The toxic manifestations one may encounter from plasmochin have been reviewed and the precautions which should be observed in the use of this drug outlined.

5. The indications for the use of combined quinine-plasmochin in the treatment of vivax malaria are discussed.

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VENOUS THROMBOSIS AFTER INFUSION WITH GELATIN SOLUTIONS
CONTAINING MERCURIAL PRESERVATIVES*

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IN a clinical study⁷ designed to test the suitability of certain gelatin solutions as plasma substitutes, the only untoward effect encountered was the frequent occurrence of thrombosis of the injected veins. This reaction both was undesirable and restricted seriously the number of veins available for repeated infusions. Because of the high incidence and considerable extent of venous thrombosis it seemed likely that the solutions contained a thrombosing substance.

The present study indicates that mercurial preservatives were the probable cause of this reaction.

A. Observations on Patients. Methods and Case Material. Eighty injections of 5 different gelatin solutions were made in 39 patients. With the exception of 4 injections of 500 to 700 cc. each infusion was 1000 cc. Before administration the fluid was warmed to room temperature in a water bath. Fluid was administered by gravity in from 80 to 120 minutes through a standard venoclysis set† employing a 19 gauge needle. No technical difficulties were encountered.

The 39 patients ranged in age from 20 to 84 years, the majority being over 50 years. They had various chronic diseases such as rheumatoid arthritis, thrombo-angiitis oblit-

erans, Laennec's cirrhosis, chronic glomerulonephritis and cerebral arteriosclerosis. Of these patients, 34 were injected with 2 or more preparations at suitable intervals to make certain that differences in response were not attributable to differences in patient material.

Five minor febrile reactions, 3 with chill, occurred during the first 18 injections. It was believed that these may have been due to insufficient preparation of the equipment. Therefore, more elaborate care was taken in cleansing glassware and tubing. Following this precaution‡ no further febrile reaction occurred.

Results. In general the infusion of gelatin solutions was well tolerated. No significant changes occurred in body temperature, pulse, respiration or blood pressure during or after the infusions, other than the 5 febrile reactions previously noted.

Thrombosis was observed in 27 of 80 veins injected with gelatin solutions. The lesion was apparent within 24 hours of injection, the veins becoming hard and cordlike. The hardened vein extended for distances of 3 to 30 cm. from the site of injection. Occasionally the vessel wall seemed thickened near the site of injection although it remained patent. In such

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Research Service, First (Columbia) Division, Goldwater Memorial Hospital.

† Upjohn Infusion Set, supplied by Upjohn Company, Kalamazoo, Mich.

‡ The procedure adopted was as follows: Dismantling of separate parts of equipment, washing with green soap, soaking in green soap for 20 minutes, rinsing in running tap water for 1 hour, rinsing in triply distilled water, packaging, and autoclaving at 15 pounds for 20 minutes.

instances the veins were not classified as thrombosed. Veins were classified as thrombosed when venepuncture showed them to be bloodless. Repeated venepuncture on an appreciable number of these vessels after several months showed the veins to be occluded. However, in some cases the veins were blood-containing after a few months. With 3 exceptions the thrombosed veins were painless and non-tender. In these 3 instances the patients had complained of pain along the course of the vessel during the infusion. No clinical evidence of embolism from the thrombosed sites was observed.

Table 1 shows the incidence of thrombosis after intravenous injections with 5 different gelatin solutions, 4 of which contained mercurial preservatives.

B. Observations on Rabbits. PRODUCTION OF THROMBOSIS. The thrombosing action of phenyl mercuric borate in 3 gelatin preparations and in 10% glucose solution was tested in rabbits. Control groups were injected with 10% glucose and with 1 gelatin preparation free of the mercurial preservative. A single dose of each preparation, equivalent to 20 cc. per kilo body weight, was injected into the marginal ear vein of the rabbit. The site of injection was then examined daily for the appearance of thrombosis. On the 4th day after injection biopsy of the site of injection was obtained by punching out a circular section of the ear, 1 cm. in diameter. Tissues were fixed and examined histologically.

The results (Table 2) correspond to the

TABLE 1.—INCIDENCE OF THROMBOSES AFTER INTRAVENOUS INJECTIONS OF GELATIN AND GLUCOSE SOLUTIONS

Preparation*	Preservative	(mg./100 cc.)	No. injections	No. thromboses
Upjohn B20610-27 . . .	Phenyl mercuric borate	4 0	16	12
Upjohn B20610-45 . . .	Phenyl mercuric borate	1 0	10	5
Knox P7-20	Phenyl mercuric borate	2 5	12	5
Knox PM10-20	Merthiolate†	3 4	15	5
Knox PX10-20	None		27	0
10% glucose in water . . .	Phenyl mercuric borate	4 0	7	5
10% glucose in water . . .	None		7	0

* The Knox preparation was characterized by high viscosity and the Upjohn by low viscosity.

† Mercury content equivalent to phenyl mercuric borate 2.5 mg. per 100 cc.

The 2 Upjohn preparations were essentially alike except for differences in concentration of the preservatives. The 3 Knox preparations were also alike but for differences in the preservatives. Table 1 shows that thromboses occurred frequently after injection with gelatin solutions containing either phenyl mercuric borate or merthiolate, whereas no thromboses followed injection with a gelatin solution (Knox PX10-20) containing no preservative. This effect of the mercurial preservatives was likewise demonstrated in control studies employing 1000 cc. of 10% glucose. Five of 7 patients had extensive thromboses after injection with 10% glucose containing phenyl mercuric borate. By contrast none of 7 patients receiving 10% glucose alone had subsequent thromboses.

observations on patients. Thromboses occurred frequently with preparations containing mercurial preservatives, whereas only 1 of 12 injections with non-preserved gelatin was followed by thrombosis. However, unlike the experience with patients, the intravenous injection of 10% glucose produced thrombosis in 4 of 6 instances. The most severe and extensive thromboses followed the injection of 10% glucose containing phenyl mercuric borate in concentration of 4 mg./100 cc. In 4 instances gangrene of the surrounding tissues appeared 2 weeks after the injections.

Thrombosis was apparent within 24 to 72 hours of the time of injection. The microscopic appearance of sections taken 4 days after injection is described as follows: Thrombi consisted of fibrin, platelets, coagulated red blood cells and widely

scattered leukocytes with faded nuclei. The thrombotic elements formed masses which projected into the lumen or lined the endothelium like a membrane. Veins containing thrombi were widely dilated, and many revealed interrupted elastic lamellæ. About the walls of several veins were dense edema, fibrin precipitates and slight infiltration of lymphoid, mononuclear and rare polymorphonuclear leukocytes. A few instances of perivascular hemorrhage were also found. No necrosis or leukocytic infiltration of the vessel wall was seen.

different concentrations was added to the gelatin solution in 2 groups of rabbits, and in another group sodium thiosulfate was added.* The results are shown in Table 3.

Thrombosis occurred in 8 of 10 veins injected with Upjohn gelatin solution B20610-27 containing phenyl mercuric borate, 4 mg./100 cc. The same incidence was obtained with this preparation in a previous series (Table 2). The addition of either sodium thiosulfate or of methionine to the gelatin solution appears to give some degree of protection against

TABLE 2.—INCIDENCE OF THROMBOSIS IN RABBIT EARS AFTER INTRAVENOUS INJECTION OF DIFFERENT GELATIN AND GLUCOSE SOLUTIONS

Solution	Phen. merc. bor. (mg./100 cc.)	No. injections	No. thromboses	Av. length of thrombus (cm.)	Sequelæ
Knox PX10-20	12	1	0.60	
Upjohn B20610-27 4 0	10	8	1.85	
Knox P7-20 2 5	10	4	0.80	
Knox P7-180 2 5	10	8	1.25	
10% glucose 4 0	6	5	4.70	4 had skin gangrene
10% glucose	6	4	1.20	

TABLE 3.—THROMBOSIS IN THE RABBIT EAR AFTER A SINGLE INTRAVENOUS INJECTION OF A GELATIN SOLUTION CONTAINING PHENYL MERCURIC BORATE: PARTIAL PROTECTION WITH SULFUR-CONTAINING COMPOUNDS

Preparations	Adjuvant (mg./100) cc.	No. injections	No. thromboses	Av. length of thrombus
Upjohn B20610-27*	None	10	8	2.2
Upjohn B20610-27*	Methionine	8	4	1.1
Upjohn B20610-27*	Methionine	16	4	1.2
Upjohn B20610-27*	Na ₂ S ₂ O ₃	15	1	0.5

* This gelatin preparation contains phenyl mercuric borate 4 mg. per 100 cc.

PROTECTION AGAINST THROMBOSIS. An attempt was made to prevent the thrombosing action of phenyl mercuric borate by adding various sulfur-containing compounds to the gelatin solution. The selections of these substances was based upon earlier reports indicating a protective effect by sulfur-containing compounds in chloroform⁵ and by heavy metal poisoning.^{3,4,10} The following experiments were performed: Four series of 10 rabbits each were employed. One gelatin preparation containing phenyl mercuric borate was used throughout. One group received this gelatin preparation alone. Methionine in

the thrombosing action of phenyl mercuric borate. Differences between the control series and that receiving sodium thiosulfate are highly significant. However, in the case of methionine these differences are not of statistical importance.

Discussion. The clinical observations show that intravenous injections of gelatin solutions containing phenyl mercuric borate or merthiolate frequently lead to thrombosis of the injected vein. Other workers⁵ employing the same preparations and similar technique have observed thromboses much less often. The reason for this difference in incidence is not

* An attempt to test the effect of cystine was unsuccessful because of the insolubility of this substance in gelatin solutions.

clear. The experimental production of venous thrombosis in the rabbit ear vein tends to support our clinical findings.

The theory has been advanced that the toxicity of mercury and of arsenic may be due largely to inhibition of $-SH$ enzymes. Recently Cook^{1,2} and his co-workers have shown that phenyl mercuric nitrate depresses the activity of certain enzyme systems important in tissue respiration, and that this depressive action can be prevented by compounds containing sulfhydryl groups. The detoxifying capacity of certain dithiol compounds (BAL)^{6,9,11} for arsenic and mercury are attributed to the presence of $-SH$ groups. Such a reaction could explain the low incidence of thrombosis with the use of plasma containing this mercurial preservative and the relatively high incidence of thrombosis obtained with the use of gelatin or glucose solutions containing the preservative. Plasma, which is made up of complete proteins containing reactive sulfur groups, might be expected to protect against mercuric ions more readily than either gelatin or glucose.

However, in the present study another explanation seems indicated, since methio-

nine and sodium thiosulfate appeared to protect against thrombosis caused by phenyl mercuric borate. The protection, therefore, cannot be ascribed to the specific presence of $-SH$ groups. It seems more likely that these substances converted the mercuric ions to the mercurous form, which would render the compound less irritative. A similar reaction has been observed between mercuric chloride and sodium formaldehyde sulf-oxalate.¹⁰

Summary and Conclusions. 1. Local venous thrombosis occurred frequently in patients injected with gelatin solutions containing phenyl mercuric borate or merthiolate.

2. Venous thrombosis did not occur in patients injected with gelatin solutions free of these mercurial preservatives.

3. Similar findings were obtained in experimental studies on ear veins of rabbits.

4. The addition of sodium thiosulfate or methionine to gelatin solutions containing phenyl mercuric borate appeared to give partial protection against the thrombosing action of this mercurial preservative.

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DISSEMINATED COCCIDIOIDOMYCOSIS LOCALIZED IN BONE*

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INVOLVEMENT of the bone by *Coccidioides immitis* is rare and relatively unknown in the eastern parts of the United States. With the return of our servicemen from endemic areas, every section of the country may be confronted with this disease. The organism is a specific fungus which produces a primary or initial infection usually in the form of a mild pulmonary involvement. This condition is benign, self-limited, and generally clears up within a few weeks. In many cases, particularly residents of endemic areas, this phase of the disease is asymptomatic. However, of those having the primary infection, a small percentage develop a secondary or disseminated involvement in various parts of the body. This is a very serious infection, it may come on months or years after the initial phase and usually leads to a fatal result. When the bone is involved, the clinical pattern of the disease frequently resembles a tuberculous infection and unless one is aware of this condition, the diagnosis is missed. Because of the importance of making an early and accurate diagnosis of fungus infection in the bone, the following cases are presented.

Case Reports. CASE 1. A colored male, aged 32, was admitted to Oliver General Hospital on April 10, 1945, complaining of pain and swelling of the left ankle. He gave a history that in the early part of January 1945, while stationed at Amchitka, Alaska, he slipped on the ice and twisted his left ankle. He had considerable pain and the

next morning went on sick call, where his ankle was strapped and he returned to duty. A few weeks later he began to have a throbbing type of pain and noticed swelling of the ankle. The pain gradually increased and on February 1 he went to the dispensary, where roentgenograms were taken which revealed a destructive process of the lower end of the tibia, with erosion and elevation of the periosteum. He was admitted to the station hospital where he was given a course of sulfadiazine and penicillin therapy. He received a total of 2,250,000 units of penicillin and 50 gm. of sulfadiazine without any relief.

The patient was inducted in the service from North Carolina on Jan. 22, 1942, received his basic training in Louisiana, spent 3 months in Alabama and 11 months in Phoenix, Arizona. He remained 6 months in Mississippi and 1 month in the state of Washington before being sent to Adak in the Aleutian Islands, where he remained for 11 months. From there he was transferred to Amchitka, where 1 month later he twisted his ankle. The patient states that he was in good health and felt well up to the time of the accident. In the past, he has had no unusual illnesses with the exception of an appendectomy in 1937.

A diagnosis was made of acute non-suppurative osteomyelitis involving the distal third of the tibia, of undetermined origin. As the patient had spent 11 months in Arizona where coccidioidomycosis is known to be endemic, the possibility of coccidioidal infection of the bone was considered. The patient was evacuated to the Zone of the Interior for study and treatment. During the middle of March, while he was in the

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process of being transferred from Alaska to this hospital, incision and drainage were performed and later a short leg cast was applied.

Upon admission the patient appeared to be slightly undernourished but not acutely ill. He had a short leg cast which extended from the toes to just below the knee joint. The cast was soft, somewhat discolored, and had a foul odor. Upon removing the plaster there were 2 fairly large granulating wounds, 1 over the inner and the other over the posterior aspect of the ankle joint. There were also 4 small draining sinus tracts, and the ankle was swollen, especially over the inner and posterior sides. The skin over

(Fig. 2). A number of specimens were taken from the wound for culture and guinea pig inoculation. The entire area was then cleaned and a new cast was applied.

Laboratory Examinations. Red blood cells, 3,190,000; white blood cells, 8850; hemoglobin, 64% (neutrophils, 65; lymphocytes, 29; monocytes, 3; eosinophils, 1; stabs, 2). The urine was acid and had a specific gravity of 1.018; albumin and sugar were negative. The blood sedimentation rate was 56 mm. at 1 hour. The smears and culture taken from the wound disclosed *Proteus vulgaris* and *Staph. aureus*. The Kahn and Wassermann reactions were negative. Smear, cul-



FIG. 1.—Note the large, irregular wound with multiple sinus tracts over inner and posterior aspects of ankle and foot.

the inner aspect was red, distended and had a shiny appearance. There was considerable purulent discharge over the posterior wound, while over the anterior aspect of the ankle there was a watery, serous exudate. The entire ankle was very tender to touch and all motions were considerably restricted and painful (Fig. 1).

Roentgenograms were taken of the ankle, disclosing a large egg-size area of rarefaction over the lower end of the tibia just above the articulating surface. There was an extensive periosteal proliferation over the inner and posterior aspects of the lower end of the bone. The ankle joint appeared free

and guinea pig inoculation of the material were all negative for *Mycobacterium tuberculosis*. Smears were negative for the actinomyces group. On culture, a fungus grew out which resembled a common contaminant.

On May 4, an incision was made over the draining sinus tracts exposing the bone where a defect was found which was filled with a grayish granulating tissue. A number of specimens were removed for culture and pathologic examination.

The pathologic report of the biopsy material disclosed on microscopic section giant cells and at rare intervals single spheroid bodies.

One or 2 of these bodies were found in the necrotic margins of the tissue. Diagnosis: "Tissue granulation with complicating secondary infection. The presence of rare spheroid bodies raises the question of fungus infection."

The patient's general condition remained unchanged. There was still considerable purulent discharge which required changing the plaster cast every 2 or 3 weeks. Complement fixation studies on this patient's serum, completed in June by Dr. D. S. Martin at

wound and the presence of an enlarged, tender femoral lymph gland, the patient was taken to the operating room on July 18 and under general anesthesia the gland was removed for study. The wound over the lower end of the tibia was enlarged and the cavity in the lower end of the tibia was thoroughly curetted and the posterior ledge of bone was resected. Several bone and tissue specimens were sent to the laboratory for examination. The report of this material is as follows:

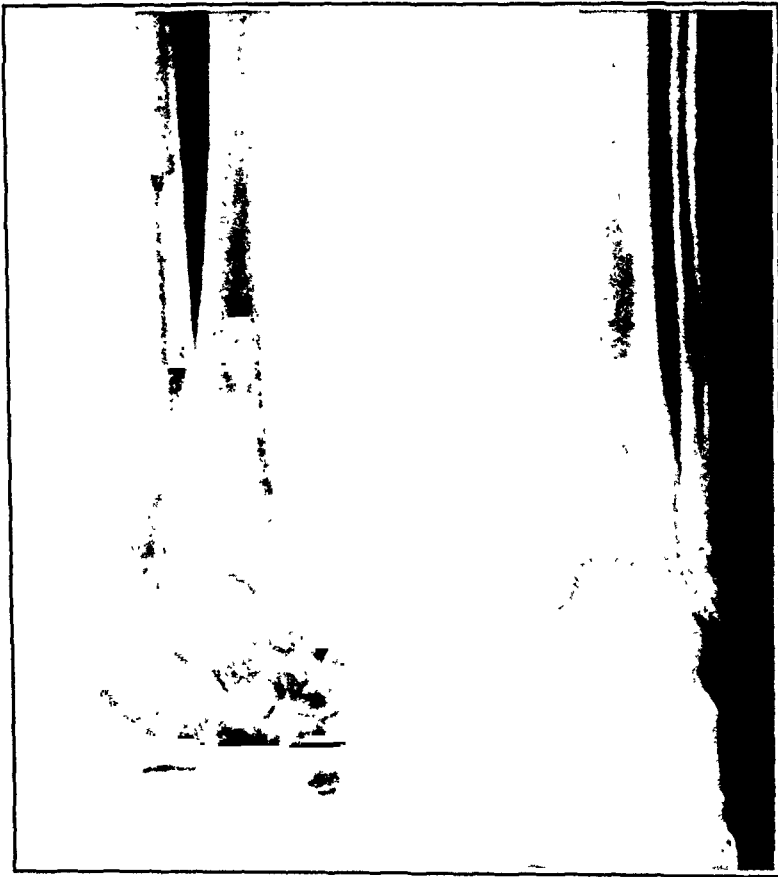


FIG. 2. - Roentgenograms showing an area of rarefaction over the lower end of the tibia, with marked periosteal proliferation over the inner and posterior aspects of the bone.

Duke University, were positive for coccidioidomycosis in a dilution of 1:64 and negative for blastomycosis.

On July 12, the patient was skin-tested with 1:100 and 1:10 coccidioidin and gave a reaction which was interpreted as moderately severe. At this time a large, painful femoral lymph gland was noted on the left side. The chest was negative, and his physical condition was satisfactory. Because of continued profuse drainage from the

Specimen A: Lymph Gland. Gross appearance: Plum-sized lobulated matted mass of nodes. Gross section of entire mass shows a thin capsule distortion of cortex and medulla. Whole mass is streaked red, white and yellow.

Microscopic studies: Distortion of follicular architecture has occurred in this group of matted nodes. Germinal cells of the follicles in many fields appear to have increased numerically and where this has oc-

curred, lymphocytes have been compressed into a circumferential mantle. Reticulum cell proliferation has occurred in the inter-follicular tissue (pulp). The tissue is vascular and reticulum cells in scattered fields take part in a tubercle-like reaction. In 1 field several mononuclear whorls are conjoined and central caseation necrosis occurs. No double contoured spherules were observed.

Specimen B: Curettings. Gross appearance: Blood-drenched soft tissue and ragged fragments of bone.

Command Laboratory for further study: (A) material from a deep necrotic focus in the bone (end of tibia); (B) curettings from the same areas as "A"; and (C) wedge of tissue from the surface of the granulating wound.

The general procedures followed in the study of the above specimens were: 1. direct microscopic examination; (2) culture of the material on (a) blood agar plates, (b) Sabouraud's dextrose agar plates, and (c) Smith's synthetic medium for *Coccidioides*

TABLE 1.—MYCOLOGIC STUDIES ON CASE 1

Material used	Direct observations	Cultural studies			Direct animal [†] inoculation studies		
		Media used	Growth	Animal [†] inoculation results	Material used	Observations	Organism recovered by culture
"A" Material from deep necrotic focus	Immature spherules seen	Blood agar	+	Typical lesions, spherules seen	"A" Untreated	Mouse died in 24 hrs. from acute bacterial peritonitis	No cultures made
		Sab.* agar	+	Typical lesions, spherules seen	"A" Concentrated by CuSO ₄	Mouse died in 24 hrs. from acute bacterial peritonitis	No cultures made
		Smith's medium	+	No mouse inoculated			
"B" Curettings	Immature spherules seen	Blood agar	+	No mouse inoculated	"B" Untreated	Mouse died in 24 hours from acute bacterial peritonitis	No cultures made
		Sab.* agar	+	Typical lesions, spherules seen	"B" Concentrated by CuSO ₄	Mouse died after 10 days; typical lesions and spherules observed	Culture isolated killed another mouse
		Smith's medium	+	No mouse inoculated			
"C" Wedge of tissue	"Spherules of coccidioides or blastomycetes not seen." [†]	Blood agar Sab.* agar Smith's medium	— — —		"C" Untreated	Mouse still living after 4 months	

* Sabouraud's dextrose agar.

[†] Report from Pathology Department, Lawson General Hospital.

[‡] Animals inoculated by intraperitoneal injection of saline suspension.

Microscopic studies: In this section of curettings from the deeper granulomatous focus in bone, the tissue reactions are similar to those described above with actual tubercle like masses, single and conjoined, with and without giant cells studding the tissues everywhere. The giant cells are enormous and the constellation of nuclei are centric, polar and peripheral. In 1 field and actually within the body of one of these giant cells, there is a spherule with double contour capsule. It contains several rounded bodies (endospores) adherent to the inner surface of this doubly refractile limiting membrane.

The following parallel material obtained at this operation was forwarded to the Mycology Section of the Fourth Service

immittis; (3) mouse inoculation with (a) untreated materials, and (b) materials concentrated by the copper sulfate method of Smith.

The results of the laboratory procedures just outlined are given in Table 1. They prove conclusively that the localized lesion involving the lower third of the left tibia was caused by *C. immittis*. While the cause of the infection was indicated by the immature spherules in the biopsy material of the first examination (May 4) and by the results of the serologic test made in June, it was proven by the isolation of the organism from the material removed from the lesion itself.

In the attempt to follow the course of the infection, a second specimen of serum was

collected on August 4 and sent to Duke University for examination. A doubtful reaction in the undiluted serum was obtained. Inasmuch as the antibody titer had dropped from a positive reaction in a 1/64 dilution in June to a doubtful reaction in the undiluted serum in August, it was felt by some that a good prognosis was suggested.

The patient's condition gradually improved. He was confined to absolute bed rest and his ankle was immobilized in a plaster cast which extended from his toes to just below the knee. On July 24 he was started on potassium iodide drops. The blood sedimentation rate on August 13 was 35 mm. at 1 hour. Vaccine treatment was started on Aug. 25, 1945. The first dose was 0.1 cc. of a 1:10,000 dilution of coccidioidin vaccine. The injection was given subcutaneously, 3 times weekly, and increased with each dose. By September 19 the sedimentation rate was down to 19 mm. On October 1 the patient developed an acute Vincent's infection of the left tonsillar region, which cleared up in 3 days with penicillin treatment.

His leg cast was removed on October 12 and roentgenograms were taken. The wound over the medial and anterior aspects of the ankle was closing in and was one-third the size noted 1 month earlier. Most of the sinus tracts over the back of the ankle were healed. Roentgenograms disclosed increased density of the bone with evidence of new bone production and healing of the bone cavity. The lower end of the tibia appeared smooth (Fig. 3). A new cast was applied and the patient was permitted to be up on crutches. Vaccine treatment was continued and on October 25 he was given 0.2 cc. of a 1:100 dilution of coccidioidin. The sedimentation rate on October 25 was down to 12 mm. at 1 hour.

At the present time the patient feels well, has gained weight and runs a normal temperature. The wound appears to be healing. The infection appears to have been arrested temporarily and it is hoped that continued treatment will prevent further dissemination of the disease and will complete the healing of the present lesions.

CASE 2. A colored male, aged 22, was admitted to this hospital from overseas on Oct. 24, 1945, with the chief complaint of pain, swelling and discharge in the region of the left great toe. He gave a history that

in the early part of August 1945, while loading boxes in his Company area in Alaska, a box fell and struck his left great toe. The toe became swollen and a week later he noticed some pus over the side of the toenail. Hot soaks were applied at the Dispensary but without any improvement in his condition. On September 12, 1945, he was admitted to the station hospital at Shemya, Alaska, where the left great toe was found to be swollen and 3 draining sinuses were present, 2 on the lateral side of the toe just distal to the nail and 1 over the anterior surface. Roentgenograms taken at this time disclosed an irregular destruction of the distal phalanx of the great toe (Fig. 4). On September 18, an incision and curettage of the toe was performed under spinal anesthesia. At operation no pus was encountered and the material removed appeared to the surgeon to be chronic inflammatory tissue. The patient was given 120,000 units of penicillin prior to operation, and 10,000 units every 3 hours for 3 days afterward. The surface wounds appeared to improve and he was evacuated to the Zone of the Interior with a diagnosis of chronic osteomyelitis of the distal phalanx of the great toe.

His military history is of interest because, as in the preceding case, he spent a period of 11 months in Arizona. He was inducted into the service from Alabama on Jan. 8, 1942, and received his basic training in Texas. From June to November 1942, he was stationed at Douglas, Arizona, and while there was confined to the hospital for an undetermined fever. He later spent 1 month at Tucson, and from December 1942 to May 1943 he was at Phoenix, Arizona. He remained 6 months in Mississippi and 1 month at Seattle, Washington, before being sent to Adak in the Aleutian Islands, where he remained for 1 year and from there was sent to Attu where he stayed until May 1945, when he was sent to Alaska.

Upon admission to this hospital the patient did not appear acutely ill. There was a rather large, irregular, granulating wound over the outer and distal aspect of the left great toe. The skin edges were raised and had a grayish, dull appearance. There was a moderate amount of purulent discharge. The distal end of the toe was greatly enlarged and very tender. His general condition appeared satisfactory and the heart and lungs were clear. Blood pressure was

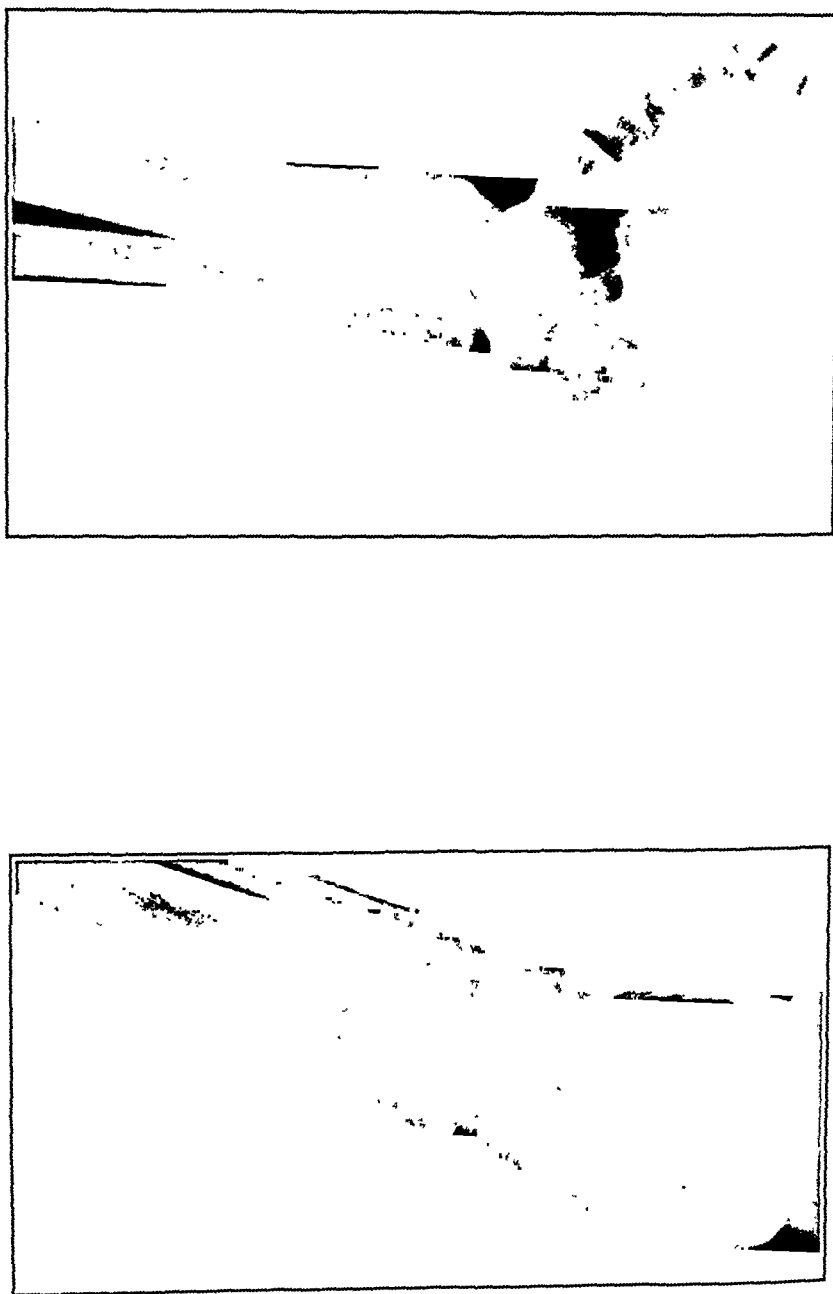


FIG. 3. *A* and *B*.—Roentgenograms taken Oct. 12, 1945, showing increased density of bone with new bone production. The posterior aspect of the lower end of the tibia is smooth and dense.

124 systolic and 74 diastolic. Liver and spleen were not palpable. Roentgenograms of the foot disclosed an area of destruction of the terminal phalanx of the left great toe. Roentgenograms of the chest and spine were negative.

Laboratory Examinations (Oct. 26, 1945). Red blood cells, 3,250,000; hemoglobin, 62%; white blood cells, 10,400; (neutrophils, 50; lymphocytes, 24; monocytes, 13; eosinophils, 10; staff cells, 3). Because of the high number of the eosinophils found, the blood count was repeated 2 days later and the count disclosed 4% eosinophils. The blood sedimentation rate

several double-contoured spherules present amid several bits of granulation tissue. Related to some of the spherules are typical Langhan's giant cells and an occasional early tubercle-like lesion, consisting of a mass of epitheloid cells with a slightly necrotic center surrounded by lymphocytes. Elsewhere the granulation tissue is heavily infiltrated by numerous plasma cells, lymphocytes and neutrophils. One tag of tissue is particularly covered by normal squamous epithelium.

Specimen B. Bloody Curettings from a Deep Focus. Microscopic studies: Essentially the same findings as in "A", with addition of a bit of calcium present on the



FIG. 4.—Roentgenogram showing rarefaction with irregular destruction of the distal phalanx of the great toe.

was 49 mm. at 1 hour. The Kahn reaction was negative. Urine was acid, specific gravity 1.014 and 1+ albumin. Skin test with coccidioidin in dilution of 1:100 and 1:10 were negative. The patient was running a moderately elevated temperature varying from 98° to 102° F. Because of continued drainage from the wound, the patient was taken to the operating room where the surrounding infected soft tissues were excised and the bone thoroughly curetted until healthy appearing bone was reached. Specimens of the soft tissue and curettings were sent to the laboratory for examination.

Specimen A. Exuberant Granulations from Wound. Microscopic studies: There are

periphery of the section. No squamous epithelium present.

The following parallel material obtained at the operation was forwarded to the Mycology Section of the Fourth Service Command Medical Laboratory for further study: (a) wedge of tissue from surface of granulating wound; (b) curettings from the deep focus in the bone (distal phalanx).

The results of the laboratory procedures are given in Table 2 and prove definitely that the localized region involving the distal phalanx of the great toe was caused by the *Coccidioides immitis*.

TABLE 2.—MYCOLOGIC STUDIES ON CASE 2

Material used	Direct observations	Cultural studies			Legend
		Media used	Growth	Animal† inoculation results	
Specimen "A" (Direct streaking of specimen)	Mature spherules with germinating endospores seen	Hormone blood agar	+	No mice injected	Direct animal inoculation studies Two mice injected; both died and typical lesions and spherules were observed; organism recovered upon culture
		Sab.* agar	+	No mice injected	
		Smith's medium	+	No mice injected	
		Special selective medium†	+	Typical lesions; spherules seen	
Swab inserted in same tube—streaked	Hormone blood agar	+	No mice injected	
		Sab.* agar	+	No mice injected	
		Smith's medium	+	No mice injected	
Washings from tube in which specimen was sent poured on plate	Hormone blood agar	+	No mice injected	
		Sab.* agar	+	No mice injected	
		Smith's medium	+	No mice injected	
CuSO ₄ concentrate	Special selective medium†	+	Typical lesions; spherules seen	
Specimen "B" (Direct streaking of specimen)	Mature spherules with germinating endospores	Hormone blood agar	+	No mice injected	Two mice injected; both died and typical lesions and spherules were observed; organism recovered on culture
		Sab.* agar	+	No mice injected	
		Smith's medium	+	No mice injected	
CuSO ₄ concentrate	Special selective medium†	+	No mice injected	

Specimen A: wedge of tissue from surface of granulating wound.

Specimen B: curettings from the deep focus in the bone (distal phalanx).

* Sabouraud's dextrose agar.

† Special selective medium in which penicillin and streptomycin are used as inhibiting agents

Details

‡ Animals inoculated by intraperitoneal injection of saline suspension.



FIG. 5.—Photomicrograph, Case 2. Note spherule with double contoured capsule.

Discussion. While the mycologic studies in both cases were similar, and the criterion for the positive identification of the culture were the same, namely the development of typical lesions and the observation of typical spherules in these lesions, one or two facts should be pointed out. First, in Case 1, the spherules as they appeared in the direct microscopic examination of the tissues were immature, "empty" double-walled structures, not showing the endospores that are normally associated with typical spherules; while in Case 2 a large percentage of the spherules observed showed the germination of the endospores within the spherule (Fig. 5). Secondly, in Case 2, the concentrated material that was prepared for direct inoculation into a mouse, grew in pure culture when streaked on culture media (although the authors of the copper sulfate method of concentration do not recommend culturing of the concentrate). Thirdly, when the infectious material was streaked upon hormone blood agar containing penicillin and streptomycin as inhibiting agents, the fungus grew in pure culture when the concentrations were 25 units of streptomycin and 6 units of penicillin per cc. of the medium. The growth on this medium was faster and more abundant than on Smith's medium.

Serologic tests on this patient were run at the Stanford University School of Medicine, and the results were as follows:

complement fixation test: 4+ in 1:64 and 2+ in 1:128; precipitin test 3+ in undiluted serum and 2+ in 1:10. The report stated "Findings indicate a disseminated coccidioidal infection (coccidioidal granuloma) which checks with finding the organism (*Coccidioides immitis*) in the tissue biopsy."

Summary. 1. Two cases of disseminated coccidioidomycosis with localized lesions in bone are presented. Both patients are colored males and give a history of having been stationed in southern Arizona for 11 months.

2. Complement fixation studies of the patients' serum were positive for *Coccidioides immitis*.

3. Microscopic examination of biopsy material disclosed immature spherules in Case 1 and typical, mature spherules in Case 2.

4. Cultures of material in both cases grew colonies of *Coccidioides immitis*. Mice inoculated with these cultures died, and at autopsy gross lesions were found characteristic of a coccidioidal infection.

5. Mice inoculated with concentrated curettings of the biopsy material died and spherules characteristic of *Coccidioides immitis* were demonstrated in the gross lesions and the organism was recovered from the animals upon culture.

Case 2 has since died as a result of disseminated lesions all over the body.

VITAMIN A AND CAROTENE METABOLISM IN THE DIABETIC AS REFLECTED BY BLOOD LEVELS

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In line with our interest in the special dietary requirements of diabetics, systematic studies of circulating vitamin A and carotene in diabetic patients were undertaken when the simple and dependable photolorimetric technique for the measurement of small amounts of vitamin A became available.^{8,16} Stated evidence in the literature, that the diabetic organism has difficulty in the normal conversion of carotene to vitamin A, was not convincing. This concept was suggested by evidence of a trend toward defective carotene "tolerance" in diabetics studied by Ralli and her associates,^{4,29} and by Heymann.¹⁴ These studies were made before it was practically possible to determine whether the tendency toward hypercarotenemia among diabetics was also accompanied by a trend toward comparatively low levels of circulating vitamin A. In later papers^{5,9} the common observation of faulty visual adaptation among diabetics was accepted as evidence of vitamin A "deficiency," an assumption that appeared untenable to us from lack of correlation between directly measured vitamin A in the circulation and visual performance in either diabetics or normals.¹⁷

In the European literature there was occasional reference to a peculiar carotene: vitamin A relationship attributed to defective handling of both the vitamin and pro-vitamin, based on the finding of excessively high levels of both in the blood stream of some diabetic patients,^{1,30} but individual data were not recorded. At

that time only a small group of continental workers were using successfully the exacting chemical determination of blood vitamin A perfected in Dutch and German laboratories,^{28,30,31} which was patently not adaptable for widespread use.^{25,27}

Our preliminary blood assays with the simplified method seemed noteworthy, mainly in a negative way, failing to reveal any impressive tendency among diabetics toward either the high carotene:low vitamin A relationship suggested by the theory of faulty carotene conversion, or the high carotene:high vitamin A mentioned by continental workers. Indeed, hypercarotenemia, with or without xanthochromia, itself seemed surprisingly inconspicuous in our general run of diabetic patients as compared with miscellaneous hospital patients whose blood we examined.

In connection with these apparent discrepancies, 2 possibilities suggested themselves: (1) That (a) the non-uniformity of diabetes, and (b) extradiabetic factors coming to be recognized as affecting available vitamin A may have been too little recognized in attempts to generalize about the diabetic's ability to handle vitamin A; and (2) that the metabolic status of diabetics in general may have improved in this as in other respects with the progress of therapy since the term "xanthosis diabetica" was introduced into the literature of the disease by Von Noorden.²⁹ We, therefore, felt it worthwhile to accumulate a sufficient body of data for somewhat detailed analysis.

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Method and Material. In the following study, levels of plasma carotene and vitamin A in 116 unselected diabetics from whom blood samples could be obtained before the beginning of hospital treatment, were scrutinized and compared with our findings for healthy control subjects of both sexes. The admission blood sample in each case was taken as representative. Later analyses made during the hospital stay are considered only by way of illustrating possible effects of treatment or other factors of contributory interest.

The chemical method which we used for determination of the vitamin A and carotene was the adaptation to plasma analysis of Dann and Evelyn's method for assay of oils⁸ previously described by one of us.¹⁶ Though the analytical procedure itself has been well standardized and individual results proved closely reproducible, there are still differences among different laboratories in the ultimate standard by which the method has been calibrated for vitamin A, and in the choice of terms in which the results are expressed. Hence *absolute* figures for the vitamin must be judged quantitatively, pending final translation, with reference to the norms established in the interpretation of the photocolorimetric measurements.

Males and females are considered separately throughout our discussion because of the widely observed sex difference in blood level of vitamin A^{3,11,12, 6,18,21,23,26} Regardless of the reason for this difference, we believe that its recognition is essential in establishing significant deviations from the normal status.

Results. *Preliminary Surveys of Data.* The *wide inter-individual variability* in the plasma vitamin levels among diabetics as compared with our findings for normal individuals is striking. A scattergraph of the values for our diabetics and of our normal controls of the corresponding sex showed that all possible types of departure from the normal range in carotene, vitamin A or both occurred in the diabetic series. Unexpectedly, the most prominent trend among the diabetics was toward *low* levels of *both* vitamin and pro-vitamin, though patients were also observed with values outside the normal range in other

directions. The problem then became one of looking into individual records to see if common denominators might be found to relate groups of patients with similar types of abnormality in their carotene: vitamin A picture.

Final Grouping of Cases and Analysis of Data. When the patients were classified for group comparisons according to factors relating to their diabetes, there was no correlation between observed plasma vitamin levels and such diabetic criteria as presence or degrees of ketosis, or increasing, decreasing or stable needs for insulin. It seemed more probable that any effect of the diabetes *per se* on the circulating vitamin supplies might well be overshadowed by factors not necessarily peculiar to the diabetic. On reviewing the individual histories the empirical scheme of case grouping shown in Table 1 seemed logical, and became the basis for further surveys of the data.

Individual data in our 116 diabetic cases are aligned according to this classification scheme in Table 2, where the wide range of blood vitamin A and carotene levels throughout the list is apparent, even within sub-groups. Our findings are summarized by groups in Table 3, where the average figures for normals are also given. In Figures 1A and 1B the data on carotene and vitamin A for both diabetics and normal controls from this laboratory* are plotted together on scatter diagrams in such a way that the diabetics and normals can be distinguished at a glance, and sub-groups among the diabetics can also be recognized.

By direct graphic comparison, 49% of our male diabetics and 47% of the females are found to have had vitamin A:carotene levels outside the extremes of the normal range when they entered the hospital. Several further generalizations can be made from simple visual inspection of the graphs: (1) Diabetics with "abnormal" vitamin A:carotene relationship are represented in all 4 quadrants of the scatter-graphs. (2) The total diabetic scatter is skewed toward the left lower quadrant,

* Reference 16 plus some additional unpublished data.

that is, in the direction of low levels of both vitamin and pro-vitamin. This trend is apparent for both sexes, but particularly for the males. (3) Symbols "1" and "2," denoting cases with active infections, stand out in this left lower quadrant of the graphs, particularly for the males. (Infections were twice as frequent among our male diabetics as among females—Table 2.) (4) The tendency toward low plasma vitamin levels noted among cases with infection is also observed for the miscellaneous or unclassified group of male diabetics (symbol "0"). (5) Diabetics with serious renal disease (symbol "X"),

xanthochromia, with a plasma carotene level above 600 γ %. Study of the individual records of these patients disclosed in 5 of the 9 factors not necessarily associated with diabetes that can contribute toward high levels of circulating carotene, *i. e.*, history of prolonged high carotene intake (patients *c*, *e* and *i*), or advanced renal disease (patients *f* and *h*, Table 5). One of these, patient *i*, affords the single instance of low circulating vitamin A with markedly elevated carotene that we had expected from the theory of defective carotene conversion in diabetics to encounter more often.

TABLE 1.—CLASSIFICATION OF 116 DIABETICS ACCORDING TO FEATURES ASIDE FROM THEIR DIABETES WITH POSSIBLE BEARING ON CIRCULATING VITAMIN A AND CAROTENE

No. cases		Identifying group symbol
Male	Female	
27	30	Miscellaneous—unclassified 0
23	11	Infected:
		Conditions frequent among diabetics 1
		Infected gangrene, infected ulcer, or osteomyelitis further complicating 1 of these 2
		Miscellaneous infections
		Infected injuries; nose or throat, head, face, teeth; urinary; abscess 2
8	12	— at:
		low fat diet; malabsorption (ex: pancreatitis) 3
		From endogenous causes 4
		Faulty storage (ex: hemochromatosis); excess use or destruction (ex: thyrotoxicosis) 4
1	4	Advanced renal disease X
59	57	

Combined in averaging

Combined in averaging

though their number is small, are invariably represented in the right upper quadrant of the graphs, showing higher than average plasma levels of vitamin and pro-vitamin.

The trends mentioned in (2) through (5) are shown numerically in the figures of Table 4, giving the proportions of each class of diabetic found to be represented in different areas of the scattergraphs.

Diabetic Carotenemia. Table 5 lists the 9 patients in our diabetic series of 116 unselected cases who on hospital admission showed frank carotenemia, *i. e.*, values exceeding the highest levels in our control series for the given sex. In the majority of these the degree of carotenemia was well within the range of levels we have seen produced by high carotene diets in non-diabetics. Only 1 patient showed

Discussion. The relatively wide inter-individual *variability* in the levels of circulating carotene and vitamin A encountered in a group of sick individuals as clinically diverse as unselected diabetics should not seem surprising, even in our present incomplete state of knowledge about metabolism of the vitamin. Wide variation also characterizes the few individual data for diabetics that we could glean from the literature.^{13,20,21,22} Without need to seek a specific diabetic anomaly one can recognize in the histories of these patients many factors capable of disturbing the vitamin A and carotene levels:

Factors that May Decrease Circulating Vitamin A and/or Carotene Levels. The effect on the plasma vitamin level of deficient exogenous vitamin supply, of conditions associated with defective absorption,

TABLE 2.—PLASMA VITAMIN A AND CAROTENE IN 116 DIABETICS ON ADMISSION TO HOSPITAL
(Key to Group Symbols in Table 1 and Figure 1)—(Continued)

MALES				FEMALES			
No.	Plasma		Notes from clinical records	No.	Plasma		Notes from clinical records
	Vit. A (I.U. %)	Carotene (% %)			Vit. A Age	Carotene (I.U. %)	
25	74	77	Old diabetes; congestive failure	29	0	0	Arteriosclerosis and Cardiovascular—CONGESTIVE FAILURE
26	74	77	Old diabetes, mild; poor control	30	67	68	
27	68	101		63	63	105	
1	58	103					
2	67	84		1	77	36	INFECTIONS COMMON IN DIABETES
3	65	47		2	62	29	Old diabetes; congestive failure; gangrene
4	58	52	Old diabetes; congestive failure; ulcer	3	57	49	Old diabetes; mild; extensive sclerosis; gangrene; cellulitis
5	61	51	Old diabetes; coma			167	Sclerotic heart disease; osteomyelitis; abscess, foot
6	61	96	Old diabetes; gangrene				
7	61	85	Old diabetes, mild; gangrene				
8	61	89	Old diabetes, fair control; gangrene; osteomyelitis				
9	50	278	Early recent diabetes; gangrene; cellulitis				
10	50	92	Old diabetes; well controlled; gangrene; cellulitis				
11	53	91	Heart disease; sclerotic; large ulcer				
12	55	36	Gangrene				
13	76	99	Mild diabetes; gangrene				
40	101	90	Mild diabetes				
	136	70	Mild diabetes, newly discovered; osteomyelitis				
	155	100	Ulcer; osteomyelitis too				
		55	Old diabetes, mild; osteomyelitis too				
14	70		Old diabetes, poorly controlled; cong. failure; ulcer				
15	30	65	INFECTIONS, MISCELLANEOUS				
16	32		Old diabetes, mild; well controlled; cong. failure; sinusitis				
17	54	156	Severe diabetes; sinusitis				
18	67	100	Old diabetes; some years; chronic infection; arthritis				
19	69	156	Mild diabetes, mod. severe; infected burn				
20	77	120	Recent infection; febrile				
21	85	103	Acute tonsillitis and pharyngitis				
22	87	103	Chronic inflamed mass in jaw and cheek; ? cause				
23	103	134	Sinusitis; infected tonsils				
24	104	98	Peritonsillar infections				
25	127	202	Sinusitis				
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KIMBLE, GERMEK, SEVRINGHAUS:

"Deficiency" Groups			"Deficiency" Groups		
3 "Exogenous"—Malnutrition			4 "Endogenous"—Hemochromatosis		
—Reducing Diet			—Reducing Diet		
1	2	3	1	2	3
66	22	22	37	59	613
103	82	296	1	178	613
151	82	310	2	44	132
151	118	372	3	89	49
151	110	112	4	81	270
151	136	201	5	96	126
151	81	24	6	96	120
151	63	210	7	69	113
151	63	210	8	64	115
151	63	210	9	70	80
151	63	210	10	76	235
151	63	210	11	82	60
151	63	210	12	87	120
151	63	210	1	125	540
151	63	210	2	139	441
151	63	210	3	144	229
151	63	210	4	168	247
151	63	210	1	37	540
151	63	210	2	37	441
151	63	210	3	56	229
151	63	210	4	51	247
151	63	210	1	37	540
151	63	210	2	37	441
151	63	210	3	56	229
151	63	210	4	51	247

X NEPHRITIS

Chr. nephritis; urea clearance 32%; secondary hypertension; sclerosis

X NEPHRITIS

Mild diabetes; pneumonia; malignant hypertension assoc. with chr. glomerulonephrosclerosis; nephrosclerosis; Old diabetes, indifferently controlled; obese; malignant nephrosclerosis; urea clearance 14 to 19%
Old diabetes; hypertensive heart disease; obese; chr. glomerulonephritis
Chronic nephritis; nephrosclerosis; early congestive failure"Deficiency" Groups
4 "Endogenous"—(Thyrototoxicosis)
Old diabetes severe, poor control; emaciated; diet mostly meat, vegs. and fruits; xanthochromia
Old diabetes, uncontrolled; Gen. A-S.; diabetes neuritis; weight loss
Diab. some years; obese; hypertensive cong. failure; reducing diet
Obese; hypertensive; reducing diet
Obese; hypertensive; reducing diet
Obese; hypertensive; reducing diet
Obese; hypertensive; reducing diet
Obese; hypertensive; reducing diet; cong. failure
Diabetes recently diagnosed; thyrototoxicosis; B.m.r. +47 to +55
Old diabetes, mild; thyrototoxicosis; B.m.r. +49
Diabetes recently diagnosed; thyrototoxicosis; B.m.r. +27
Diabetes newly discovered, mild; thyrototoxicosis; B.m.r. +80 to +90
Diabetes known 2 years, mild; thyrototoxicosis; B.m.r. +45

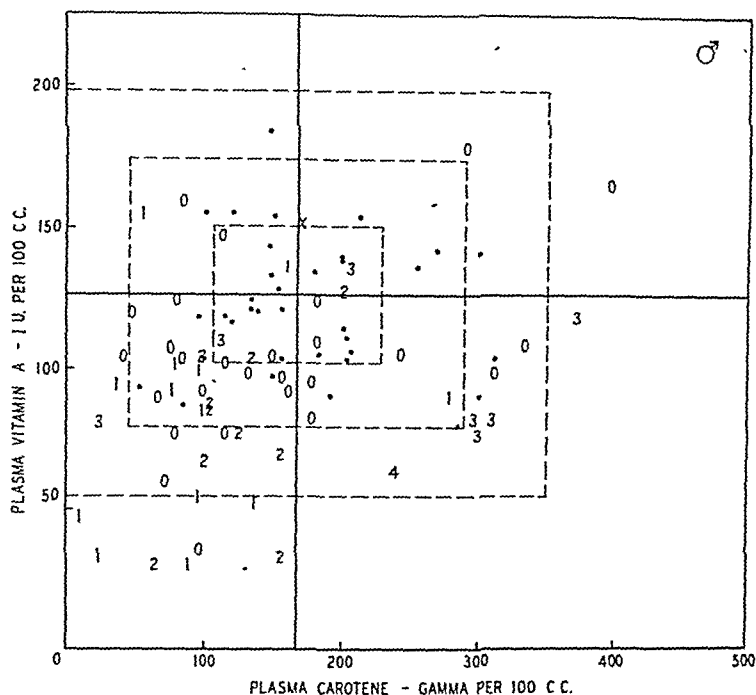


FIG. 1.—A.

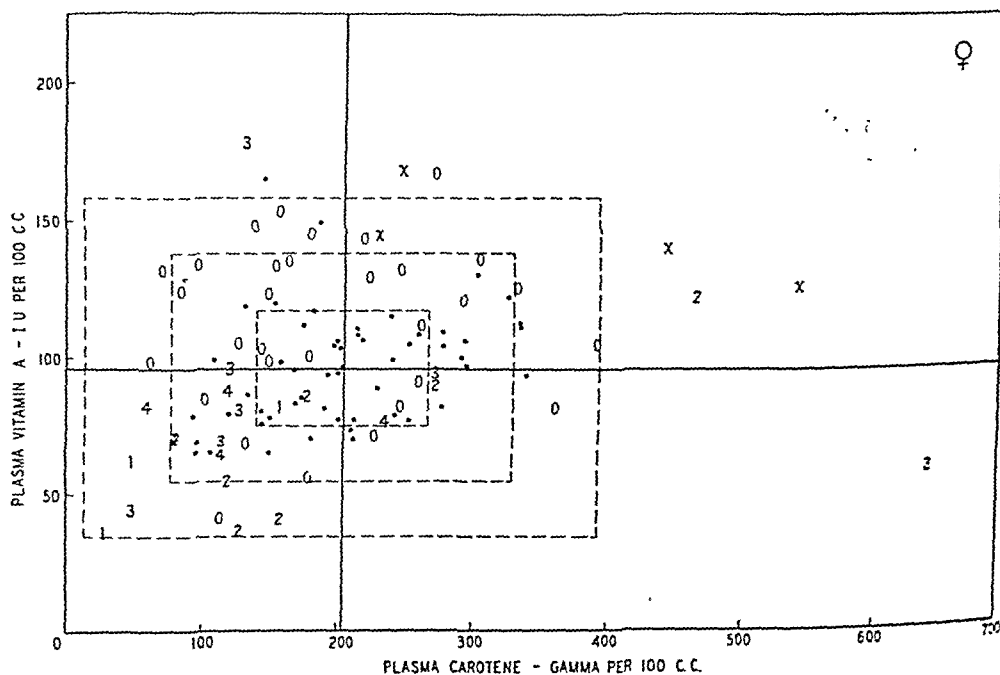


FIG. 1.—B.

FIGS. 1A and 1B.—Plasma vitamin A and carotene relationship in diabetics in comparison with normal controls of the same sex. Normal controls, 0. Diabetics: Miscellaneous—unclassified, 0. Infected cases, with: Conditions common in diabetes, 1; miscellaneous conditions, 2. Presumptively vitamin A deficient, through: Exogenous causes, 3; endogenous causes, 4. Nephritics, X. Solid Coordinates = average levels for normal controls. — — —, Broken lines define distances of 1, 2 and 3 std. deviations from averages.

and of liver disease need not be discussed here. Nor are these, of course, peculiar to the diabetic. Less generally realized in this connection, perhaps, are the possi-

bilities of fever and sepsis, and perhaps of acidosis, and low fat diet.

The association of lowered circulating vitamin A levels with factors of *sepsis*

TABLE 3.—AGE AND PLASMA VITAMIN A AND CAROTENE AVERAGES OF DIABETICS BY SUB-GROUPS OF WORKING CLASSIFICATION (TABLE 1)

Group and identifying symbol	Males					Females				
	No. cases	Av. age	Av. vit. A (I.U. %)	Av. car. (γ %)	Av. A/C ratio	No. cases	Av. age	Av. vit. A (I.U. %)	Av. car. (γ %)	Av. A/C ratio
0 Miscellaneous, unclassified	12	34	10	27
A-S and C-V, normotensive	9	55	5	59
Hypertensive	3	66	13	61
Cong. failure	3	69	2	65
All miscellaneous	27	..	105	150	0.70	30	..	113	195	0.58
1 Infections, common in diabetes	13	60	82	95	..	3	65	60	78	..
2 Infections, miscellaneous	10	36	78	124	..	8	28	75	192	..
All infections	23	49	80	108	0.75	11	38	71	161	0.44
3 Possible vit. A deficiency: Exogenous	7	30	101	271	..	7	54	88	208	..
4 Possible vit. A deficiency: Endogenous	1	45	63	240	..	5	53	76	122	..
All deficiencies	8	32	94	232	0.40	12	53	83	172	0.48
X Nephritis	1	66	151	168	0.90	4	45	144	364	0.40
Averages for normal controls			167	126	0.76			96	203	0.45

TABLE 4.—DISTRIBUTION OF PLASMA VITAMIN A:CAROTENE LEVELS OF DIABETICS BY SUB-GROUPS (Percentages of Each Group Represented in Different Quadrants of the Scattergraphs)

Group symbol and type	Males				Females			
	LLQ Low vitamin A Low carotene	LUQ High vitamin A Low carotene	RUQ High vitamin A High carotene	RLQ Low vitamin A High carotene	LLQ Low vitamin A Low carotene	LUQ High vitamin A Low carotene	RUQ High vitamin A High carotene	RLQ Low vitamin A High carotene
0 Miscellaneous	(a) 50 (b) 59	8 7	17 7	25 27	18 13	55 44	18 30	9 13
1 Infections	(a) 92 (b) 83	8 9	.. 4	.. 4	86 73	.. 9	14 9	.. 9
2 Deficiency	(a) 20 (b) 33 11	89 56	75 67	.. 8	25 25
X Nephritic	(a) .. (b)	100	100 100

(a) Cases with abnormal vitamin levels.

(b) All cases in this group.

TABLE 5.—DIABETICS WITH ABNORMALLY HIGH PLASMA CAROTENE FOUND IN UNSELECTED SERIES OF 116 CASES ON HOSPITAL ADMISSION

No. in this list	Group and Group No. (Table 2)		Sex	Age	Plasma			Notes from clinical records
					Vit. A (I.U. %)	Carotene (γ %)	A/C ratio	
a	0	18	M	53	108	334	0.32	Fairly recent diabetes, mod. severe; dehydrated; weight loss
b	3	4	M	17	118	372	0.32	Old diabetic, now in ketosis; markedly undernourished; rapid weight loss recently
c	0	20	M	59	165	396	0.42	Old diabetes, severe; high vegetable, good fat diet, adhered to for years
d	0	11	F	50	82	360	0.23	Mod. severe diabetes for several years; weight loss
e	0	21	F	55	104	390	0.38	Recent diabetes, stable; consistently high vegetable, liberal fat diet
f	X	2	F	37	139	441	0.32	Old diabetes, not adequately controlled; malignant nephrosclerosis with low urea clearance
g	2	11	F	24	122	463	0.26	Old diabetes, not well controlled; chr. susceptibility to infections; hypodermic abscesses now
h	X	1	F	37	125	540	0.23	Mild diabetes; pneumonia; malig. nephrosclerosis
i	3	1	F	37	60	643	0.09	Old diabetes, severe, inadequately controlled; emaciated; diet mainly meat, vegetable and fruits for long time; xanthochremia

and fever has been pointed out in patients with miscellaneous diseases.^{6,13,23} In *acute infections* the decrease in circulating vitamin also appears to be acute, and need not be looked upon as a sign of vitamin depletion. The temporary nature of the fall in blood vitamin levels with acute infections in children^{6,18} appears also in the cases of adult diabetics listed as patients 1 through 5 in the Appendix herewith, who happened to develop acute infections while under our observation. The sharp drop in plasma vitamin A following onset of the febrile complication, with recovery in the level paralleling clinical recovery of the patients, could suggest augmented temporary use or destruction of the vitamin because of the infectious process. Another explanation might be, as Popper and Steigmann suggest,²³ in secondary hepatic changes which temporarily inhibit release of the vitamin from the liver into the blood. Ellison and Moore¹⁰ found that liver stores of vitamin A were not reduced in patients who had died of acute infections.

The low blood vitamin levels that show up prominently among our diabetic patients with *chronic infections* common in this disease, on the other hand, can with confidence be taken to represent actual depletion of body stores of vitamin A. Moore's vitamin A assays¹⁹ in the livers of patients who had died of various types of disease did not show low liver stores in diabetics as a class. On the other hand, depleted stores were the rule in septic diseases of chronic types including osteomyelitis, cellulitis, carbuncles and septic arthritis. Our diabetics with similar infections such as often complicate this disease represent on the whole an older age range than the unclassified cases (Table 3). The low blood vitamin levels prominent in the whole infected group (symbols "1" and "2") may reflect both the long-time effects of chronic disease and the immediate effect of febrile complications. Incidentally, A/C ratios in this group average close to those for our normal controls of the appropriate sex (Table 3).

Cases 4 through 7 in the Appendix suggest that *diabetic coma* acts much like an acute infection in causing sharp but temporary decrease in the levels of circulating vitamin A, though more evidence on the degree of reversibility of this effect is needed. In view of the profound disturbance in metabolism within the liver and kidneys, among other tissues, provoked by diabetic acidosis, it is not surprising that marked decreases in circulating vitamin A should be seen in these patients, nor that these, according to some observations by Wendt,³⁰ may not always be promptly reversed when insulin therapy is first made effective.

Cases 8 and 9 in the Appendix suggest that an incidental possibility for rapid reduction of plasma vitamin A levels that could easily be overlooked in single determinations may lie in sudden shift to a *low fat diet*. These changes (and the others in the appended cases) are significantly larger than any short-time changes we have observed in following the plasma vitamin A of subjects on reasonably constant regimens.

Factors That May Increase Circulating Vitamin A and/or Carotene Levels. Two possible factors aside from exaggerated intake need to be considered in this connection: hyperlipemia and renal disease, neither of which is peculiar to diabetics. Observations by Josephs¹⁵ suggest that in conditions with *hyperlipemia* the high concentration of lipoids in the blood may retain large amounts of carotenoids in a non-utilizable state in the circulation. That no complete or simple explanation for the variable incidence of carotenemia in groups of diabetics could be hoped for in this factor, however, seemed clear early in our study. In unpublished experiments we had found that neither vitamin A nor carotene in the plasma was affected by frank lactescence after vitamin- and fat-rich meals. In preliminary studies on our diabetics, the smaller amounts of total fat in the plasma before meals was determined gravimetrically without any evidence whatever of its correlation with total

carotenoids in the same plasma. Further lipid studies of the ordinary types within our reach therefore did not seem promising.

The elevated levels of vitamin A and/or carotene in the plasma of some patients with advanced *renal disease* again suggests a possible relationship with hyperlipemia, since in some forms of nephritis all of the lipid constituents of the blood are said to be elevated.²² Sporadic reports in the literature^{6,23,32} of surprisingly high vitamin A levels in patients with nephritis are matched in our own laboratory records. These show that the highest levels we had encountered in any undosed individual were recorded and confirmed in a non-diabetic patient with nephritis. In this patient, as in 1 of our nephritic diabetics (patients X-1, F) and occasional other non-diabetic nephritics traced in our records, low levels of plasma vitamin A would have been anticipated either because of infectious processes, or dietary history. It would be interesting to know how often the factor of renal disease has been present in diabetics in whom elevated levels of vitamin A or carotene have been noted.

The high levels of vitamin A in the blood stream of the nephritic are not necessarily inconsistent with the low liver stores in this disease reported by Moore¹⁹ and others quoted by him. Baumann, Foster and Moore² have shown that certain conditions which deplete the liver of its protein stores also interfere with the hepatic storage of vitamin A, presumably by reducing the liver constituent with which the vitamin is chemically associated. Loss of protein from the body in certain forms of kidney disease might also reduce the protein necessary to store vitamin A. This factor, with liberal vitamin A in the diet and possibly elevated blood fat, could contribute to the unexpectedly high plasma vitamin levels of the nephritic.

The Concept of "Diabetic Carotenemia." There remain for further consideration certain individual cases of carotenemia among our diabetics (Cases a, b, d, g, and the extreme condition in Case i in Table 5)

and the relatively small incidence of carotenemia in our series in comparison with other studies. One factor, *poor nutritional status*, not necessarily considered dominant in our original classification of the cases, was common to 4 of these 5 patients, and could possibly be suspected in the fifth from her chronic susceptibility to infections. Inadequate nutrition in connection with poor carotene use also invites speculation from the observation that 6 of the 8 diabetics in our entire series with plasma carotene:vitamin A outside the extreme of the normal range in the direction of high carotene:low vitamin A (right lower quadrant in the 2 scatter graphs) were in 1 of the groups judged nutritionally "deficient" in our original working classification. Patient i, with the most extreme carotenemia and lowest A/C ratio in the series, had this feature in addition to a long-continued high carotene diet.

The possible importance of general nutritional status in the ability to handle carotene normally was reinforced at this point in our study by the re-reading of Conner's classical paper on carotenoid measurements.⁷ In his discussion he pointed out a prevalence of hypercarotenemia among certain non-diabetic groups—orphans and inmates of asylums—who were notably ill-nourished at the time of the first World War. This was evidently lost sight of in the search for explanation of the carotenemia seen among diabetics.

The metabolism of carotene, in which the provitamin is broken down to yield vitamin A, may prove to involve a step-wise oxidative reaction, rather than simple splitting with hydration, often assumed.* This may be but 1 of many similar metabolic processes, perhaps all of them linked with the metabolism of carbohydrate, which can no longer be properly consummated when nutrition of the organism as a whole breaks down. While total starvation leads to exhaustion of stores and very low blood levels of both vitamin A and carotene, essential malnutrition in the

* We are indebted to Dr. C. A. Baumann for this suggestion.

presence of continued intake of carotene-containing foods may result in carotene accumulation and vitamin A starvation such as we see in some diabetics. This picture seems to be growing less familiar

as time goes on, partly, it would be reasonable to suppose, as general malnutrition, deliberate or unavoidable, becomes correspondingly less frequent among diabetic patients.

CASES SHOWING POSSIBLE EFFECT OF SPECIFIC FACTORS ON PLASMA VITAMIN A

CASE 1. No. 120677, F, age 69. Mild diabetes, without ketosis, discovered on admission for cataract surgery. 15 units protamine insulin.

Date	Vitamin A	Carotene	A/C ratio	Remarks
3/2	132	70	1.9	
3/9	120	65	1.8	
3/12	Upper respiratory infection with fever
3/16	99	59	1.7	
3/23	103	59	1.7	

CASE 2. No. 225588, F, age 62. Mild diabetes of long standing, with extensive sclerosis, gangrene, cellulitis, and minimal chronic sepsis.

Date	Vitamin A	Carotene	A/C ratio	Remarks
3/20	123	41	3.0	Febrile pyelonephritis dev.
3/27	56	53	1.1	
4/3	102	48	2.1	Afebrile for 3 days

CASE 3. No. 207874, M, age 17. Moderately severe diabetes of long standing, admitted for dietary adjustment.

Date	Vitamin A	Carotene	A/C ratio	Remarks
1/26	100	82	1.2	
1/30	Developed acute tonsillitis; convalescence very slow
2/6	85	103	0.8	
2/13	78	89	0.9	

CASE 4. No. 63364, M, age 65. Long-standing diabetes; admitted in bad condition for treatment of gangrene with osteomyelitis.

Date	Vitamin A	Carotene	A/C ratio	Remarks
5/16	47	10	4.7	Toxic, comatose
5/19	Mid-thigh amputation, followed by septic temperature
5/23	28	50	0.6	
5/30	48	91	0.5	

CASE 5. No. 224948, F, age 17. Severe diabetes for 7 years, care inadequate. Healing excoriations.

Date	Vitamin A	Carotene	A/C ratio	Remarks
2/1	79	204	0.4	
2/7	81	216	0.4	
2/12	Developed acute tonsillitis
2/14	41	159	0.3	
2/23	104	106	1.0	
3/5	101	79	1.3	
3/16	96	115	0.8	Discharged; uncoöperative
4/15	54	81	0.7	Readmitted in coma

CASE 6. No. 96501, F, age 15. Diabetic 5 years.

Date	Vitamin A	Carotene	A/C ratio	Remarks
3/3	36	127	0.3	Acute cystitis; comatose
3/10	133	211	0.6	After recovery from coma, but pyuria still present

CASE 7. No. 226263, F, age 13. Recent diabetes, poor state of nutrition.

Date	Vitamin A	Carotene	A/C ratio	Remarks
4/25	56	178	0.3	Comatose
5/2	112	246	0.5	After vigorous diabetic therapy

CASE 8.—No. 220696, F, age 63. Moderately severe diabetes of long standing, with arterio-sclerosis and hypertension.

Date	Vitamin A	Carotene	A/C ratio	Remarks
12/5	148	138	1.1	
12/11	Transferred to low fat diet
12/18	96	225	0.4	

CASE 9. No. 225252, F, age 68. Mild diabetes, recent, with arteriosclerosis, hypertension; admitted for cataract surgery.

Date	Vitamin A	Carotene	A/C ratio	Remarks
2/18	124	84	1.5	Transferred to low fat diet
2/28	97	113	0.9	
3/7	69	91	0.8	

Summary and Conclusions. Among 116 unselected diabetics whose plasma vitamin A and carotene were determined by an assay method of proved dependability, all possible types of deviation from the normal range in vitamin A:carotene relationship were observed. Values for one or both substances outside the normal range for the appropriate sex were found at hospital admission in the blood of 49% of the 59 males and 47% of the 57 female patients. The predominant type of deviation in the series as a whole was low vitamin A:low carotene. This was particularly noticeable among older patients and patients with infection. Least prominent of any trend was that toward high carotene with deficient vitamin A that has been widely assumed to be characteristic in diabetes, and has been rationalized as result-

ing from a defect in the diabetic organism's ability to derive vitamin A from its pro-vitamin, carotene. Relatively uncommon in our series, also, was so-called diabetic carotenemia, considered without regard to accompanying vitamin A.

When we scrutinize individual cases in the light of our present knowledge of factors that can alter vitamin A supplies, availability, reserves, use, and the resultant blood levels, it is evident that there is no type of aberrant blood picture found among our diabetics that cannot be justified by considerations not necessarily peculiar to the diabetic, especially if general nutritional failure (less frequent than it used to be among diabetics) is added as a possible cause for inefficient use of ingested carotene.

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RECURRENT FATAL HEMOLYTIC ANEMIA ASSOCIATED WITH GROSS LIVER DAMAGE AND SPLENOMEGALY

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THOUGH much has been done in recent years to explain the circumstances under which intravascular hemolysis occurs, this remains a confused corner of medicine. The following rare case is reported as it may be of assistance in filling in the picture of this group of diseases.

Case Report. The patient, a 30-year old Arab policeman stationed in Baghdad, was admitted to the Royal Hospital on July 20, 1944, complaining of weakness and pallor for 1 month. There was no history suggestive of malaria, bilharziasis, dysentery or venereal disease, nor had he previously been jaundiced. He was married, his wife and 3 children all being in good health; his wife had had no abortions. There was no history of jaundice in the family. During the last month he had noticed that he was becoming paler, losing strength, suffering from breathlessness on slight exertion and from headaches and attacks of giddiness. He was not aware of having had any hemorrhage and there had been no change in the color of his stools or urine. There was no pruritus.

On examination he was found to be a well-built man, very pale, with puffiness of the face and slight edema of the feet. The sclera were icteric though this was not marked enough to have been previously noted by the patient. The liver edge was palpable $\frac{1}{2}$ inch below the costal margin, being smooth and not tender. A hard smooth spleen could be felt extending 2 inches below the left costal margin. Temperature, 37°C .; pulse rate, 90; blood pressure, 118/60. After a week in hospital he discharged himself but was re-admitted 6 weeks later when he stated that shortly after leaving hospital he started to have bouts of fever which came at irregular intervals and were not associated with rigors or severe sweating. His strength had decreased progressively and his eyes had become more yellow.

On re-admission, apart from an increase in pallor and jaundice, the findings on physi-

cal examination were the same as at the time of his previous admission. Temperature, 37.5°C .; pulse rate, 100. The urine contained bile pigments but no albumin, sugar or abnormal microscopic constituents. The stool was normal in color and on microscopic examination showed nothing abnormal. The serum gave a delayed direct van den Bergh reaction; the Wassermann reaction was negative. No malarial parasites were found in the blood films on repeated examination and a blood culture was sterile. The blood group was A. The fasting gastric juice contained no free HCl; after histamine, 10 ml. N/10 HCl per 100 ml. The sternal bone marrow showed an intense normoblastic reaction. The blood findings are given in detail in Table 1, and in Figures 1 and 2. They are those of a gross normochromic, normocytic anemia. The red cells showed anisocytosis and polychromatophilia but no spherocytosis or macrocytosis. The white cell count and differential count were normal. A red cell fragility test gave partial hemolysis in 0.45% and complete hemolysis in 0.36% sodium chloride solution.

Course of Illness (Fig. 1). Perhepar (5 ml.) was given intramuscularly twice weekly and a ferrous sulfate mixture. On Sept. 21, 1944, he was transfused with 500 ml. citrated Group A blood which had been cross-typed at room temperature and found to be compatible. A mild reaction consisting of shivering, rise in temperature and abdominal pain occurred towards the end of the transfusion. On September 28 a second transfusion of 500 ml. citrated blood was given from a different Group A donor who had been found to be suitable by cross-typing. After 100 ml. had been transfused there was a rigor, the temperature rose to 39°C ., there was severe abdominal pain, vomiting and purgation. The typing and cross-typing were checked and found to be correct. In the course of the next 48 hours the temperature settled to its original value but on the 5th day after the transfusion it rose

suddenly to 39.6° C. and remained high for 3 days, during which time the jaundice became much more intense, the urine became dark with bile, and the serum gave a strong immediate direct van den Bergh reaction. Reference to Figure 1 shows that the blood findings are such as would be produced by a brisk hemolysis.

On September 10 blood was obtained from the patient and both donors and the following experiment carried out: 5 tubes were set up, 3 contained the patient's serum, which had not been previously chilled, with, respectively, the patient's red cells and the red cells of the 2 donors; and 2 contained the sera and red cells of the 2 donors. After incubation overnight, all the tubes containing the patient's serum showed complete hemolysis, while the tubes containing the donors' sera showed none.

pleural adhesions on both sides and some congestion of the base of the right lung. The heart was large and flabby and there were milk spots on its anterior surface. The abdominal cavity contained no free fluid. The liver weighed 2700 gm., was soft, congested, bile-stained and showed fatty change. No obstruction was to be found in the biliary tract. The spleen weighed 1400 gm., was firm in consistency, dark in color, with slight perisplenitis. The kidneys showed cloudy swelling. The bladder, ureters, prostate, pancreas, adrenals and digestive tract showed no abnormalities.

Histologic Examination (see Fig. 3). *Liver.* There is generalized cloudy swelling and gross fatty degeneration, the majority of the cells containing large fat globules. The degeneration is greatest in the center of the lobules. The sinusoids are dilated. The

TABLE. 1—BLOOD PICTURE ON 22 x 44

Red blood cells	1,700,000 per c.mm.
Hemoglobin	5.24 gm. per 100 ml.
Red cell volume	14.5%
M.C.V.	85 cu.u.
M.C.H.C.	36%
M.C.H.	31 yy
Reticulocytes	15%
White cells	10,500 per c.mm.
Neutrophils	59%
Basophils	1%
Eosinophils	1%
Myelocytes	1%
Lymphocytes	31%
Monocytes	7%
Normoblasts	4 per 100 WBC
Total serum protein	6.5 gm. per 100 ml.
Serum albumin	3.6 gm. per 100 ml.
Serum globulin	2.9 gm. per 100 ml.

Splenectomy was advised but refused, and treatment was continued with liver extract and iron. At first there was rapid improvement in both the anemia and the jaundice until, by the middle of October, a condition similar to that before the transfusion was reached. On November 1 the patient's serum was again incubated with his own red cells and those of the same 2 donors; no hemolysis occurred in any of the tubes. Soon after this the red cell count and the hemoglobin started to fall, at first slowly and then more rapidly, being accompanied by an increase in the jaundice. Finally the patient died on November 21, when the red cells were below $\frac{1}{2}$ million per c.mm.

Postmortem Examination. The cadaver was markedly jaundiced. There were small

parenchyma is bile-stained and a few bile thrombi are to be found in the smaller ducts. The Küpffer cells are hypertrophied and they and the liver cells contain much iron pigment.

Spleen. There is marked thickening of the trabeculae and generalized fibrosis replacing the reticulo-endothelial cells. In some areas the sinuses are packed with ghost-like erythrocytes and in others they are comparatively empty. The lymphoid tissue generally is greatly reduced in amount, the Malpighian corpuscles being practically unrecognizable. Hemosiderin is scattered irregularly throughout the tissue. There is hyaline degeneration of the vessel walls.

Kidney. There is generalized cloudy swelling of the tubular epithelium. The tubules are dilated, some containing bile-

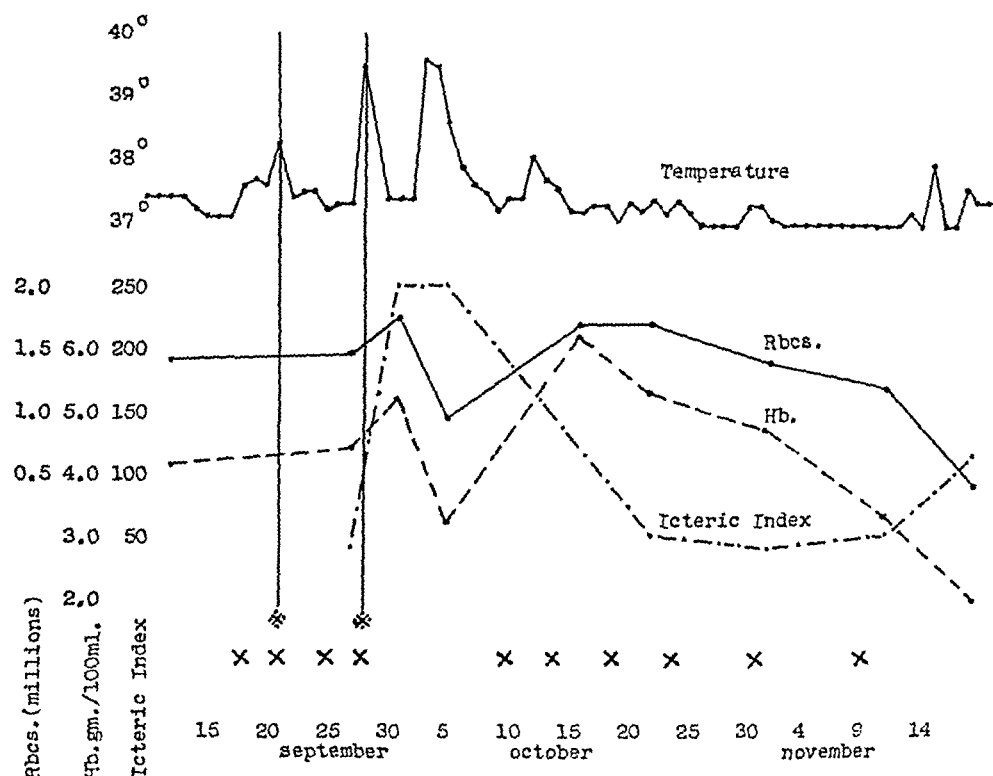


FIG. 1.—Temperature, red cell count, hemoglobin %, icteric index and treatment. X, 5 ml. perhepar injected. * 500 ml. blood transfusion.

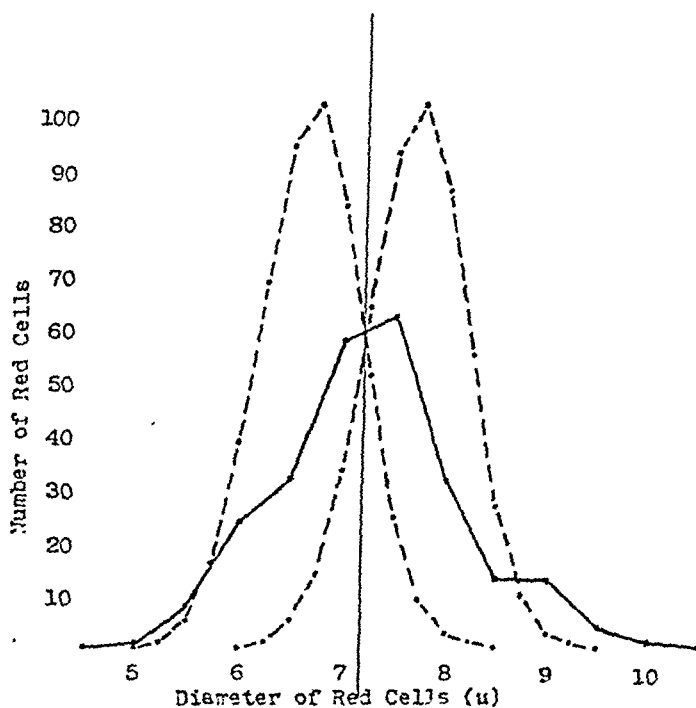


FIG. 2.—Red cell distribution curve. Mean diameter, 7.3μ ; standard deviation, 0.9μ ; coefficient variation, 12.2%; mean thickness, 2.1μ ; diameter-thickness ratio, 3.5

stained casts. There is no obvious congestion. The glomeruli are shrunken and cellular and the capsular space is dilated but no red blood corpuscles are present in it. In a few glomeruli the capsular space contains amorphous albuminous matter. There is no indication of any glomerular or interstitial nephritis and no proliferation of the cells of Bowman's capsule. There is a heavy deposit of hemosiderin in the tubular cells and no proliferation of interstitial tissue.

Bone Marrow. This shows marked normoblastic hyperplasia and congestion. The sinuses are packed with ghost-like erythrocytes. There is no obvious proliferation of leukoblastic elements.

tive, nor to which subgroups they belonged. There was, however, a gross degree of anemia before any transfusion had been given and the fatal hemolysis was not preceded by a transfusion. Rapid hemolysis started 12 days after the first transfusion and 5 days after the second. If the hemolysin found during this crisis had been due only to an incompatible transfusion it would probably have persisted¹; instead it had disappeared 3 weeks later. Acquired intra-group incompatibility does not therefore alone explain this anemia nor the presence of the hemolysin.



FIG. 3.—A, Spleen. Sinuses packed with ghost-like red cells. $\times 150$.
B, Liver. Sinuses empty. $\times 150$.

Comment. The case is one of hemolytic anemia accompanied by gross fatty degeneration of the liver and marked histologic changes in the spleen. No evidence of bacterial or protozoal infection could be found and enquiry failed to reveal contact with any hemolytic chemical substance. The hemolytic process showed periods of exacerbation and remission. During a period of exacerbation hemolysins were demonstrable in the serum; during a period of remission they were not.

One hemolytic crisis followed a transfusion and the possibility of intra-group incompatibility needs to be considered. It was not possible to determine whether the bloods used were Rh positive or nega-

That the jaundice was partly toxic in origin was shown, during life, by the presence of bile in large amounts in the urine and by the direct van den Bergh reaction. The histologic appearance of the liver explains its failure to excrete bile pigments.

Farrar, Burnett and Steigman⁴ report a case similar in many respects. The history and course of the disease were similar and the blood picture only differed in that it showed some degree of macrocytosis. The descriptions of the liver and spleen correspond and a hemolysin was demonstrated in the serum. In this case the condition was aggravated by transfusion, the hemolysis and jaundice being so marked 6 days after the first transfusion that

these were discontinued. This, together with the experience in the case described, suggests that transfusion may play some part in precipitating a hemolytic crisis. Splenectomy was followed by rapid and complete recovery.

The relationship between the hemolysis and the liver damage needs further consideration. Was the liver damaged as a result of having to deal with the large quantities of the products of red cell destruction or was it damaged by the hemolysin or some other toxic substance in the circulation? Fatty degeneration of the liver may occur in hemolytic anemias^{2,3} but it is rarely extensive and there may be severe hemolysis, as in the hemoglobinurias and in Lederer's anemia, with little or no liver change. Two other pertinent cases have been found in the literature in which hemolysis was accompanied by a severe toxic hepatitis. In Lovibond's case⁵ this was ascribed to a coincidental streptococcal septicemia, while in Ayer and Kammer's case¹ of hemolytic anemia, caused by the transfusion of Rh positive blood

into an Rh negative recipient, the condition was thought to be due to an intercurrent catarrhal jaundice. In the present case and that described by Farrar, Burnett and Steigman, the most likely explanation seems to be that the spleen was responsible for the formation of a hemolytic and hepatotoxic substance or substances. Splenectomy offers the best chance of survival; and as in both cases transfusion was followed by a severe hemolysis, this may be dangerous and the possibility of hemolysis occurring several days after the transfusion should be borne in mind.

Summary. 1. A fatal case of recurring attacks of hemolytic anemia is described.

2. During a hemolytic crisis hemolysins were demonstrable in the serum.

3. The hemolytic process was accompanied by a hepatitis.

4. At postmortem gross fatty degeneration of the liver and splenomegaly with marked histologic changes in the spleen was found.

I am indebted to Dr. Shawkat Zahawi of the Royal Faculty of Medicine, Baghdad, for the post-mortem report on the histologic preparations; and to the Department of Parasitology of the London School of Hygiene and Tropical Medicine for the photographs.

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CONVALESCENCE FROM SURGICAL PROCEDURES

III. THE RELATION OF NITROGEN BALANCE AND BLOOD VOLUME TO
ABNORMALITIES OF THE CIRCULATION*

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IN previous communications^{12,13} several studies of convalescence after surgical operations were reported. After operation the resting circulation was often somewhat abnormal, and the circulation in the upright position was usually grossly abnormal for a short time. In addition, transient abnormalities in the response to mild exercise could be demonstrated after operation. Finally, while studies of nitrogen balance confirmed the finding that the body lost nitrogen in the convalescent period when the patient was on the usual postoperative routine, this loss of nitrogen could usually be prevented if the nitrogen and caloric intakes were kept high enough.⁸

The present study was made possible by the development of a new and portable type of ballistocardiograph by Rawson, Hervey and Lilly.⁷ Two of these instruments, 1 for the horizontal and 1 for the vertical position, were installed in a room adjacent to the surgical ward. This improvement in our equipment permitted us to test patients when their clinical condition was poor, a type of study previously avoided because of the long trip necessary before the surgical patients reached the old stationary ballistocardiograph located at the medical end of the hospital. Therefore, the results reported in this study were

obtained on a group of patients, most of whom were convalescing from operations far more serious than in the previous studies.^{12,13} Abnormalities of the heart and circulation were found to be very much more common during convalescence from the more severe operations.

Our studies of the circulation of these patients, together with knowledge of their nitrogen balance from the study which was running concurrently, made it possible to answer the question whether the maintenance of a positive balance was accompanied by objective improvement in postoperative condition. We found that this was so.

Also, estimates of the blood volume of many of our patients, performed by Dr. C. E. Koop,⁶ made it possible to study the relation of the blood volume to the form and size of the ballistocardiogram and to the cardiac output as calculated from it. We found a significant relation between blood volume and cardiac output and also between blood volume and the degree of abnormality of form of the ballistocardiograms.

New Apparatus and Its Use. Significant features of the new horizontal and vertical ballistocardiographs designed and construct-

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Pennsylvania.

ed by Rawson, Hervey and Lilly are shown in Figures 1 and 2. Lilly's capacitance manometer⁷ was used to amplify the movements. The calibration was entirely linear. The advantages of these instruments over our old apparatus were: (1) they were portable, and (2) records could be taken without darkening the room.

taken and the resulting deflections of the base line recorded the calibration on each ballistocardiogram.

When the record was studied, the deflection of the base line caused by the weight was determined and the measurement of the altitude of the "I" and "J" waves was adjusted accordingly. The "area" method

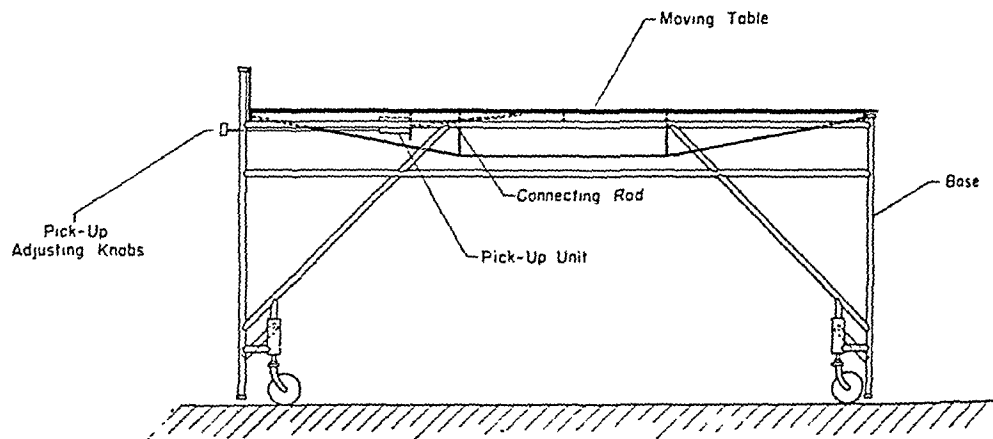


FIG. 1.—A new ballistocardiograph with electrical amplification. This instrument was designed and constructed by A. J. Rawson, J. P. Hervey, and J. C. Lilly in the Johnson Research Foundation of the University of Pennsylvania. The electrical circuit has been described⁷. The new ballistocardiograph consists of a table weighing 35 pounds constructed of duraluminum. This table is suspended from a tubular steel frame by a flexible ribbon of phosphorus bronze at each corner. The effective length of the suspension is 2.5 cm. at the head, 7.5 cm. at the foot of the table. The frame is provided with large swivel castors so that it can be readily moved. The castors can be raised so that the base rests on rigid steel legs while the ballistocardiograph is in operation. One leg is adjustable to compensate for irregularities of the floor. The moving table is rigidly connected with the condenser micrometer pick-up unit. Relative motion of the condenser plates changes the timing of a high frequency oscillator circuit whose output drives a General Electric Co. oscillograph. A Cambridge Instrument Company Electrocardiograph camera is used to record the ballistocardiograms. The over-all sensitivity is adjusted to give a deflection of 1 cm. for a force of 280 gm. The natural vibration frequency of the ballistocardiograph table, loaded with 150 pounds of rigid weight, is 11.8 per second.

A vertical ballistocardiograph using the same method of recording was constructed also. Except for the new recording system its structure did not differ from the instrument described before¹⁴. Its natural vibration frequency is 15.5 per second when supporting 150 pounds of rigid weight.

The advantage of these new instruments are: (1) they are portable; (2) they can be used without darkening the room; (3) they are rugged and simple to operate.

The procedure for taking records differed but little from that used on the older machines. About 15 minutes was required to warm up the tubes of the amplifier, and this was done while the subject was resting on the apparatus.

When the instruments were made, the springs were adjusted until the deflection of the light spot for a given weight approximated that used in the original ballistocardiograph; but as the amplification was not always constant, a calibration had to be made for every record. We always added a known weight while the picture was being

was used to calculate cardiac output. We no longer believe that the factor for aortic size,¹⁵ included in the original formula, is needed to calculate cardiac output. In this investigation we are concerned chiefly with the changes in cardiac output in single individuals so that this factor cancels out and does not enter into the great majority of the results reported here.

Procedure. The 67 patients were all operated upon by members of the Surgical Services of the Hospital of the University of Pennsylvania. The anesthesia varied with the type of operation. Local, spinal, cyclo-

propane and ether were employed. Dihydro-beta-erythroidin was employed occasionally in 50 to 300 mg. doses because of its curare-like action.

The operations performed can be divided into several groups: (1) 11 craniotomies, (2) 16 operations on the gall bladder and biliary tract, (3) 13 operations on the stomach for ulcer or carcinoma, (4) 14 operations on the large bowel, chiefly resections and

followed by a ballistocardiogram. Those who could stand then arose and stood on the vertical instrument for 2 minutes, after which blood pressure and a ballistocardiogram were again taken.

To obtain a record as soon as possible after operation the patient was stopped on the way from the operating room, and a ballistocardiogram was taken in the horizontal position. Some patients were still

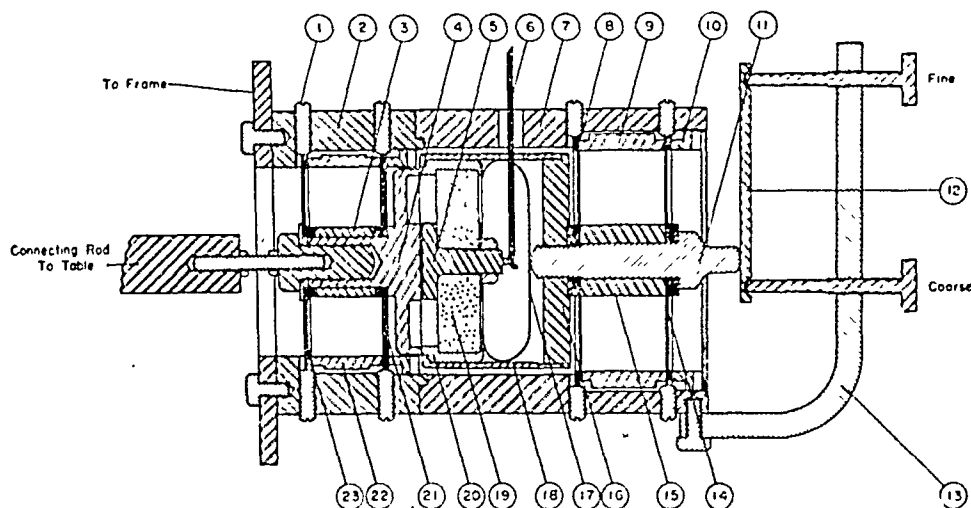


FIG. 2.—Condenser micrometer pick-up unit. The pick-up unit is mounted on the tubular steel base. The measuring springs (21 and 23) and moving condenser plate (4) are linked to the bed by a connecting rod fitted with stout lengths of $\frac{1}{2}$ inch diameter phosphorus bronze rod at each end. These give sufficient transverse flexibility so that only longitudinal movements of the bed are transmitted to the condenser. Horizontal motion of the bed is restrained by the measuring springs (21 and 23). These are of such stiffness that a force of 280 gm. produces a movement of approximately 6 microns. The gap between the moving condenser plate (4) and the reference plate (5) is adjusted by changes in the coarse and fine adjusting knobs which work through the lever (12) and the stud (11) and deflect the springs (8 and 14).

Since both condenser plates are mounted on disk springs, they always move parallel to each other. The reference plate (5) is spring mounted within the shield (16 and 18) so that excessive movement of either plate will not strain the insulator (19) and destroy the adjustment of the plates.

The numbers refer to the following: (1) alignment screws, (2) measuring spring case, (3) and (22) measuring spring spacers, (4) moving condenser plate, (5) reference condenser plate, (6) condenser lead, (7) reference plate and spring case, (8) and (14) reference plate springs, (9) and (15) reference spring spacers; (10) reference spring clamp nut, (11) reference spring stud, (12) adjustment lever, (13) adjusting screw bracket, (16) and (18) reference condenser plate mount, (17) reference condenser mount spring, (19) reference condenser plate insulator, (20) insulator shell, (21) and (23) measuring springs.

anastomoses, (5) 6 herniorrhaphies and (6) a miscellaneous group of 6, containing 1 each of the following: thyroidectomy for non-toxic goiter, exploratory thoracotomy for carcinoma, splenectomy, removal of renal calculus, excision of pancreatic cyst, and removal of functioning islet tumor of the pancreas.

All patients were tested at least once before operation. After they had lain on the horizontal ballistocardiograph for 15 minutes, their blood pressure was taken and this was

unconscious at this time, and none of them were asked to stand.

Whenever the patients' condition permitted it, they were tested 1, 2, 5 and 10 days after operation, and just before discharge from the hospital. Most of the patients were unable to stand in the early postoperative period. In all, 393 ballistocardiograms were taken on 63 patients.

In the immediate postoperative period the nitrogen balance was studied in 36 of our patients by a team under the direction of

Dr. C. E. Koop and Dr. Cecilia Riegel. These tests ran for the first 5 days following operation, and the results will be described in detail elsewhere.⁹ The figures recorded are for this 5 day period unless otherwise noted. The blood volume was estimated by the method of Gregerson,⁴ as modified by Gibson and Evans,³ and by Evans *et al.*,¹ using

is used only in the statistical sense of indicating that the probability is less than 5 in 100, that the results obtained are due to chance.

Results. *On Abnormal Ballistic Forms Occurring After Operation.* In our study of convalescence after herniorrhaphy¹³ the

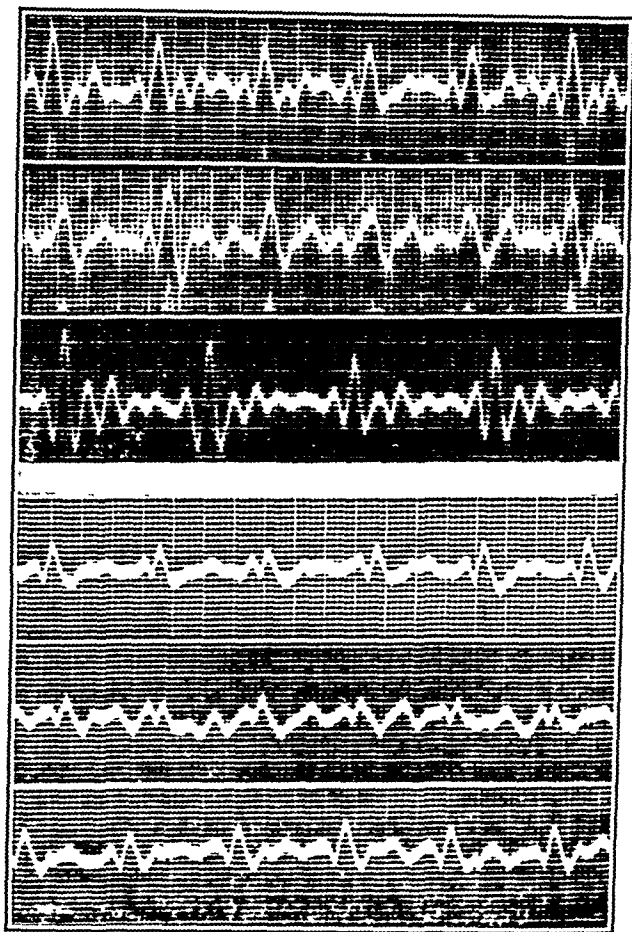


FIG. 3.—Ballistocardiograms before and after operation. Top 3 records of G. M., aged 48; before, 6 days, and 15 days after an extensive bowel resection for carcinoma of the splenic flexure. Last 3 records of V. S., aged 36; before, 4 days, and 9 days after cholecystectomy.

the blue dye T-1824. This was done in 34 of our cases before and 5 or 6 days after operation. These determinations were made by Dr. C. E. Koop, who will report the results in more detail elsewhere.⁶

A statistical analysis was performed on the data and significance was judged by the criteria of Fisher.² As is usual with papers from this laboratory, the word significant

form of the ballistocardiograms remained normal, and this permitted us to calculate the change in cardiac output due to the operation in every case. In the present study the situation was very different, for not only were the operations of a much more serious kind, but also the patient had often suffered from a prolonged illness

before operation. In addition, this study contained far more persons in the older age group than was the case in the previous study. Probably as a result of these factors, abnormalities in the form of the ballistocardiogram were encountered frequently before and after operation. Figure 3 gives 2 examples of the latter. Present knowledge does not permit the calculation of cardiac output from abnormal curves.

The first step in the analysis of these records was to search for abnormal forms

the abnormal records of this group the "I" wave was always less than one-quarter the area of the "J" wave.

In Grade 2 were placed records with complexes characterized by an abnormally small or disturbed "I" wave similar to that of Group 1, together with abnormalities of "J" also, the latter peak being flattened, notched or otherwise distorted. Figure 4 shows several examples.

In Grade 3, judged the most severe, were placed records described before¹¹ as of the late downstroke type and character-

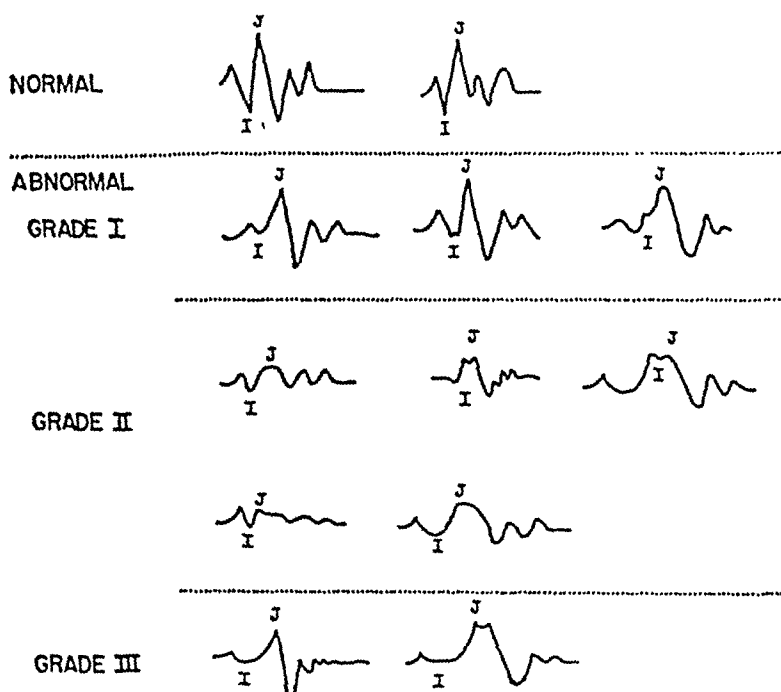


FIG. 4.—Normal and abnormal forms of the ballistocardiogram. All the forms drawn here were encountered in this study.

and identify their type by help of calipers. In most records all of the abnormal complexes found were of the same type; in all records a single type predominated. Empirically, we set up the series of abnormal forms illustrated in Figure 4. In Grade 1, which we judged to be the least severe, were placed records showing an abnormal "I" wave. This wave was either distorted in form or abnormally small in relation to the size of the "J" wave. Normally the area of "I" averages about one-half that of "J," and their time duration on the base line is almost equal. In

ized by a shallow rounded "I", a broad low "J" with its peak well back in systole, and with a sharp JK downstroke the most conspicuous line on the record. An example is drawn in Figure 4. We encountered only a few records of this type.

The second step in the analysis of these records was to note the frequency of the abnormal complexes. In many records the form varied with respiration, the smaller complexes being abnormal, while the larger remained normal. In such records we assumed that the degree of

abnormality was proportional to the percentage of abnormal complexes.

A third criterion in judging the degree of abnormality was the amplitude of the record in terms of the calibration. In the normal records this is reflected in the estimation of cardiac output, but in these abnormal records this estimation was not attempted. Nevertheless, knowing by experience the amplitude to be expected in normal subjects we did not hesitate to judge unusually small complexes as abnormal; and when, in a series of records on one subject, the amplitude diminished without an increase of rate, we judged that cardiac function was becoming more abnormal.

Therefore, by means of these 3 criteria—frequency, type, and amplitude—we attempted to judge the degree of the abnormality of our patients, especially to ascertain whether they were improving or becoming worse. This was not difficult because the 3 criteria usually varied in the same direction. When the records of any patient were compared, it was usually an easy matter to decide whether he was improving or losing ground. We have recorded our decisions by dividing the patients with abnormal ballistocardiograms into 3 classes according to the degree of abnormality, and these classes appear in the tables as Roman numerals. When the patient's ballistocardiogram changed for the worse, we assigned it to a higher class. This method, we believe, recorded changes in 1 patient's circulation with reasonable accuracy. To judge the relative degree of abnormality in 2 patients whose records are different is a more difficult and uncertain matter unless the difference is very striking. The situation is complicated by the multiple criteria of abnormality, and the form of abnormal ballistocardiograms varies so greatly that they cannot be divided into 3 uniform classes. Hence, under each of the 3 classes recorded in the tables, more than one type of abnormal record has been included.

As these factors made it difficult to classify the records with exactitude, great

care was taken that unconscious bias did not enter in. The records were presented to the senior author for reading in such a way that he knew nothing of the nature of the case under consideration. In this way they were classified without prejudice or preconceived ideas.

In compiling Tables 1 and 2 the degree of abnormality was not considered, and the records were classified as normal or abnormal. Table 1 shows the frequency of the abnormal records before and after the different types of operations. It will be seen at once that, while the records of many patients are abnormal before operation, this number doubles in the immediate postoperative period, to decline to less than the original number at discharge. Obviously in these seriously ill patients the effect of the operation is to produce a temporary abnormality which manifests itself by the abnormal ballistocardiogram. It should also be noticed that operations on the colon and biliary passages seem especially prone to produce transient ballistic abnormalities after operation while the cranial operations produced only 1, a difference which is significant.

In Table 2 the same data with additional cases from the miscellaneous group are classified according to age. Here it will be seen that the ballistic abnormalities present before operation were found chiefly in patients over 40 years of age and that it is largely in this group that they persisted until discharge. In the immediate postoperative period abnormalities may be found in all age groups. However, when only those cases that were normal before operation were considered, we were unable to demonstrate that age had an influence on the tendency to become abnormal after it.

Effects of the Metabolic Condition on the Circulation. One of our chief objectives was to compare the condition of the patients who remained in nitrogen equilibrium after operation with that of those whose balance became negative. Therefore, we asked ourselves whether the ballistic form became abnormal more fre-

quently in the group in negative nitrogen balance. In Table 3 the data have been arranged according to the nitrogen balance, and it will be seen at a glance that ballistic abnormalities occurred far more frequently after operation in the group whose balance was negative. This difference is statistically significant.

sion that nitrogen imbalance played a part could be safely drawn. We, therefore, resorted to the method of paired experiments.

In pairing the data, first consideration was given to the type of operation. Cases falling into the miscellaneous group were not used, so both members of every pair

TABLE 1.—FREQUENCY OF ABNORMAL FORMS OF THE BALLISTOCARDIOGRAM IN DIFFERENT TYPES OF OPERATION

Type of operation	Time relation to operation					
	Before		1 to 10 days after		At discharge	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Colonic	6	7	1	12	7	6
Gastric	8	4	7	6	7	4
Cranial	9	2	8	3	10	1
Biliary	13	4	4	12	13	3
Hernial	4	2	1	5	4	1
Total	40	19	21	38	41	15

TABLE 2.—FREQUENCY OF ABNORMAL FORMS OF THE BALLISTOCARDIOGRAM IN DIFFERENT AGE GROUPS

Age	Time relation to operation					
	Before		1 to 10 days after		At discharge	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Over 70	0	1	0	1	0	1
60-69	3	5	1	7	3	4
50-59	2	9	2	9	2	9
40-49	16	5	8	13	18	2
30-39	12	1	8	5	11	1
20-29	8	0	5	3	7	0
10-19	3	0	1	2	2	1

However, a careful study of the problem made us hesitate to accept these results as proof of our point. Some of the cases, mostly those in the craniotomy group, had been handled differently from the others. They had been placed on a high nitrogen diet before operation to permit them to accumulate stores of nitrogen; after operation they drew on these stores but did not exhaust them in most instances and so remained in positive cumulative balance. The different types of operation also were not evenly distributed in the series. In addition, the group in positive nitrogen balance averaged a little younger than those in negative balance. Since the data in Tables 1 and 2 suggest that age and the type of operation are factors in the ballistic abnormalities, further analysis of the data was needed before the conclu-

had had operations falling within one of the groups mentioned before. The second criterion was age, the pairs being arranged so that their ages were as close as possible. A third criterion—sex—was used in some cases, but it was considered less important than age and never sacrificed to it. By this means 17 pairs were obtained, their nitrogen balance being -135 and $+114$, $+25$ and $+66$, $+18$ and $+52$, $+81$ and $+106$, -50 and -43 , -24 and $+11$, -48 and -16 , -46 and -1 , -46 and -1 , -38 and -31 , -36 and $+21$, -9 and 0 , -21 and $+25$, -13 and $+40$, -12 and -1 , -12 and -3 , -13 and $+66$.

For each individual the effect of the operation on his ballistocardiogram was ascertained, and the result was scored as follows: If a patient's record was normal before operation and became abnormal

Grade 1 after it, the score was recorded as -1. If the form changed from normal to Grade 2, the score was -2. If a Grade 1 abnormality before operation was followed by Grade 2 after it, the score was -1, etc.

in better metabolic condition developed less ballistic abnormality after operation than did his mate. In only 2 pairs was the reverse true. In 4 pairs there was no difference. This result is not quite significant, but the chances are about 92 in

TABLE 3.—DEGREE OF BALLISTIC ABNORMALITY COMPARED WITH THE CUMULATIVE NITROGEN BALANCE

Nitrogen balance, gm	Type of operation	Age	Form of ballistocardiogram		
			Before operation	After operation	On discharge
+114*	Cranial	35	Normal	Normal	Normal
+106*	Colonic	45	Normal	I	Normal
+81*	Gastric	48	II	III	Normal
+66*	Gastric	33	Normal	Normal	Normal
+66*	Cranial	47	Normal	Normal	Normal
+52*	Cranial	23	Normal	Normal	Normal
+40	Colonic	52	I	III	I
+25	Colonic	69	Normal	I	Normal
+25*	Cranial	43	Normal	Normal	Normal
+21	Gastric	55	Normal	Normal	Normal
+18*	Cranial	15	Normal	Normal	Normal
+11	Hernial	44	Normal	Normal	Normal
+0	Gastric	65	Normal	Normal	Normal
-1	Gastric	38	I	Normal	Normal
-1	Miscellaneous	27	Normal	Normal	No test
-1	Cranial	27	Normal	Normal	Normal
-3	Colonic	15	Normal	II	Normal
-9	Gastric	75	II	III	I
-12	Colonic	55	I	III	II
-12	Colonic	48	Normal	III	Normal
-13	Colonic	50	III	Normal	Normal
-13*	Gastric	32	Normal	III	III
-16	Cranial	47	I	II	Normal
-21	Colonic	66	I	III	I
-24	Hernial	47	I	II	Normal
-31	Gastric	49	Normal	Normal	Normal
-36	Gastric	62	No test	III	III
-38	Gastric	55	Normal	II	I
-43	Hernial	28	Normal	I	Normal
-46	Cranial	21	Normal	Normal	Normal
-46	Gastric	44	Normal	Normal	Normal
-48	Cranial	45	Normal	Normal	Normal
-50	Hernial	19	Normal	II	I
-62	Cranial	42	II	III	III
-135*	Cranial	38	Normal	I	Normal

I, II, III = Grade of abnormality.

* Patient was placed on a high nitrogen diet before operation, and the balance recorded is cumulative for 5 days before and 6 days after operation. In all other patients the nitrogen balance is cumulative for the first 6 days after operation only.

The nitrogen balance of 1 of each pair was naturally more positive than the other, and this difference was usually large. Therefore, to find the effect of the metabolic difference on the ballistocardiogram we subtracted the score of the patient in the better nitrogen balance from that of his more abnormal mate. The results were striking. In 10 pairs the individual

100 that a poor metabolic condition is associated with the development of ballistocardiographic abnormalities after operation.

Using the same pairs we asked ourselves whether the difference in metabolic condition was reflected by any difference in the effect of the operation on pulse rate or blood pressure. The statistics were worked

out, and we were unable to demonstrate that the metabolic difference was associated with any significant difference in the behavior of pulse rate and blood pressure after operation.

We also sought to test the condition of those in better nitrogen balance by comparing the duration of hospital stay after operation in the pairs of cases mentioned above. On the average the patient in the better metabolic condition remained in the hospital 2 days less than his mate, but the data scattered widely, and this result is not significant. In studying this data we became aware that completely extraneous factors, such as the number of hospital beds available, had an effect on the duration of the patient's stay.

Two of the patients paired died while in the hospital, and both were in worse metabolic condition than their mates. In 1 case death followed a pulmonary embolus. In the other, tuberculosis of the cecum was present, and the course was progressively downhill. The number of deaths is too small to make the result significant.

The surgeons in charge of these patients believed that those kept in positive nitrogen balance did better than the others. A case supporting that viewpoint is given in detail below.

B. W., aged 45, was operated upon twice for carcinoma of the colon. The results obtained in the horizontal position were all normal the day before the operation, the pulse rate being 69; the blood pressure, 124/74; and the cardiac output -9% . On her return from the operating room after a Rankin obstructive resection of the transverse colon performed under spinal anesthesia, despite the fact that she had received 600 cc. of blood and saline while on the table, the blood pressure had fallen to 88/62; the pulse rate to 51, and the cardiac output to -26% . After operation the patient developed a slight fever, maximum 101.6°F. , which lasted 18 days. At the next test 6 days after operation, blood pressure, pulse, and cardiac output had returned to normal, but the form of the ballistocardiogram had become abnormal, the smaller "J" waves of

the respiratory cycle being flattened or notched and broader than normal. The form of the record returned to normal before the second operation, but probably because of the fever the cardiac output was elevated to $+34\%$ at this time.

In anticipation of the second operation she was given large feedings for 5 days to build up her nitrogen stores. The colostomy was closed 17 days after it had been established. She stood this second operation better than she had the first, in spite of the fact that the resection was easily performed while the closure was long, tedious and traumatic. The pulse rate, blood pressure, and cardiac output were essentially unchanged after it, and the form of the ballistocardiogram remained normal. However, on the 6th day after the resection she was given 500 cc. of 6% gelatin intravenously as a preliminary to a tilt table test. She was placed on the ballistocardiograph 3 hours after this test. At this time the abnormality of form noted after the first operation was found to have returned. This abnormality did not persist, and in 2 subsequent tests before discharge all our findings were normal.

It would appear that this patient withstood the second of 2 comparable procedures better than the first. The second operation was performed after her nitrogen reserve had been built up while the first had not been preceded by any nutritional preparation.

Further Studies by Means of Paired Observations. Needless to say, pairing the experiments in the manner described before does not dispose of all the variables. Indeed, since no 2 patients are ever exactly alike, the control of a clinical investigation is never ideal. We tried to answer other questions by making other pairs.

We had data in 9 cases that had been fed large amounts before operation. We used only the postoperative nitrogen balance in each case, neglecting the increased stores of nitrogen which had resulted from the extra feeding. Searching among the cases not fed extra amounts before operation we selected 9, each of whose postoperative nitrogen balance agreed closely with 1 of the first 9. The closeness of the

agreement is shown by the following list, the figures being grams of nitrogen lost in the 5 day period after operation, the value found in the patients given extra food coming first: -68 and -62, -47 and -46, -54 and -50, -34 and -31, -25 and -24, -24 and -21, -16 and -16, -12 and -13, -8 and -9. We then compared the effect of operation on the circulation of each pair. In 4 pairs the case given extra food before operation showed less abnormality after it than did his mate; in 1 pair the reverse was true; in 3 the effects of operation were similar. This fails to demonstrate that the cases given extra food before operation were significantly better, but the odds are about 3 out of 4 that it is so.

age systolic blood pressure, unchanged early in convalescence, diminished slightly in the late stages. Average cardiac output was never significantly different from the value found before operation.

To our disappointment most of these patients could not stand, and many of these who could had so much tremor that the ballistocardiograms were worthless, so the few results we obtained in the standing position seem unworthy of record.

Relation of Blood Volume to Circulation. Our figures for blood volume, calculated from plasma volume and hematocrit, were expressed as percentage deviation from the expected normal by means of the monogram given by Gibson and Evans.¹ Our data must be divided into 2 parts.

TABLE 4.—CHANGES IN CARDIAC OUTPUT, PULSE RATE, AND BLOOD PRESSURE FOUND AFTER SERIOUS OPERATION

Pulse rate per min. . .	Mean	σ^2	n	Sig.	SERIOUS OPERATION															
Systolic B.P., mm. Hg	+20	18	34	Yes	1 to 5 days postop.				5 to 10 days postop.				Before discharge							
Diastolic B.P.	-11	19	35	Yes	Mean	σ^2	n	Sig.	Mean	σ^2	n	Sig.	Mean	σ^2	n	Sig.				
Cardiac output, % of	-5	10	36	Yes	+10	12	42	Yes	+7	13	54	Yes	+4	12	37	Yes				
av. normal . . .	+11.3	35	18	No	0	17	44	No	-6	17	56	Yes	-6	17	42	Yes				
					+2	9	43	No	-1	8	56	No	-1	10	41	No				
					+6	3	15	27	No	-1.3	24	36	No	-2.7	23	26	No			

The means given are averages of the differences between the values found before operation and the values found after it. Thus on returning from the operating room the pulse rate averaged 11.3 per cent higher than it had before operation.

Postoperative Changes in Cardiac Output, Pulse Rate, and Blood Pressure Found After

The means given are averages of the differences between the values found before operation and the corresponding values found after it. Thus on returning from the operating room the pulse rate averaged 20 beats per minute faster than it had before operation.

Postoperative Changes in Cardiac Output, Pulse Rate, and Blood Pressure. In the records of 44 patients the ballistic form remained normal, and from these we calculated cardiac output in the usual manner. The analysis of these data, as well as those concerning pulse and blood pressure, have been recorded in Table 4. This table provides a picture of the average reaction of the resting circulation to severe operative procedures. All these results were secured with the patients resting in the horizontal position.

On return from the operating table the average pulse rate was significantly elevated, and average blood pressure was significantly diminished. The cardiac output varied widely; and although the average showed an increase of 11%, its significance was not demonstrated.

As convalescence proceeded, the average pulse declined towards normal, and aver-

The relation between blood volume and cardiac output, as calculated from the normal ballistocardiograms, is illustrated in Figure 5. Using all the values obtained before and after operation, there is significant correlation between blood volume and cardiac output per minute, the correlation coefficient being 0.355 while any value over 0.29 is significant. On the other hand, the relation is obviously not a very close one, and in single persons a change in blood volume was often not followed by a corresponding change in cardiac output.

Figure 6 shows the relation of blood volume to the abnormal ballistocardiograms. The relation is quite striking, the most abnormal records occurring altogether in persons with small blood volumes. The mean of this group is significantly different from the other means. We investigated the relation between

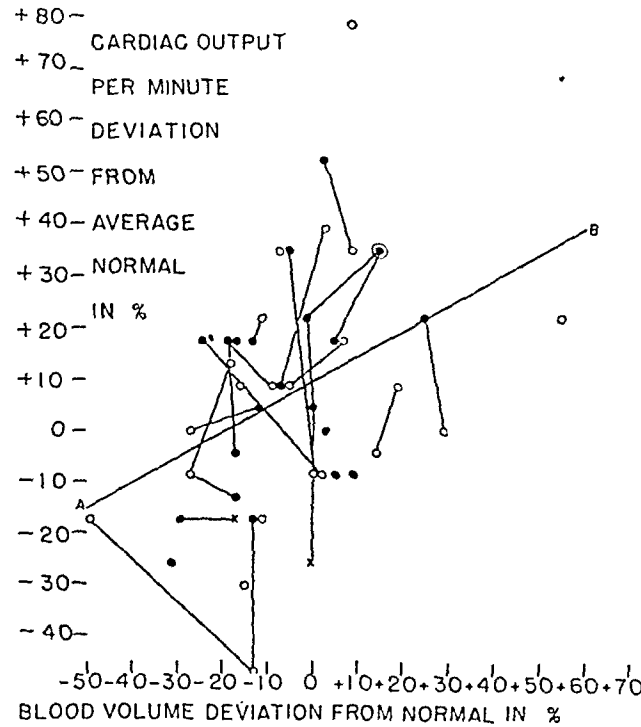


FIG. 5.—Relation of blood volume to cardiac output. Both values are expressed as percentage deviations from the expected average normal for each subject. Line AB is the calculated regression y on x , its equation $y = 0.107x + 25.06$. Circles represent values obtained before operation, dots those secured after it. Values obtained on the same patient are connected by lines.

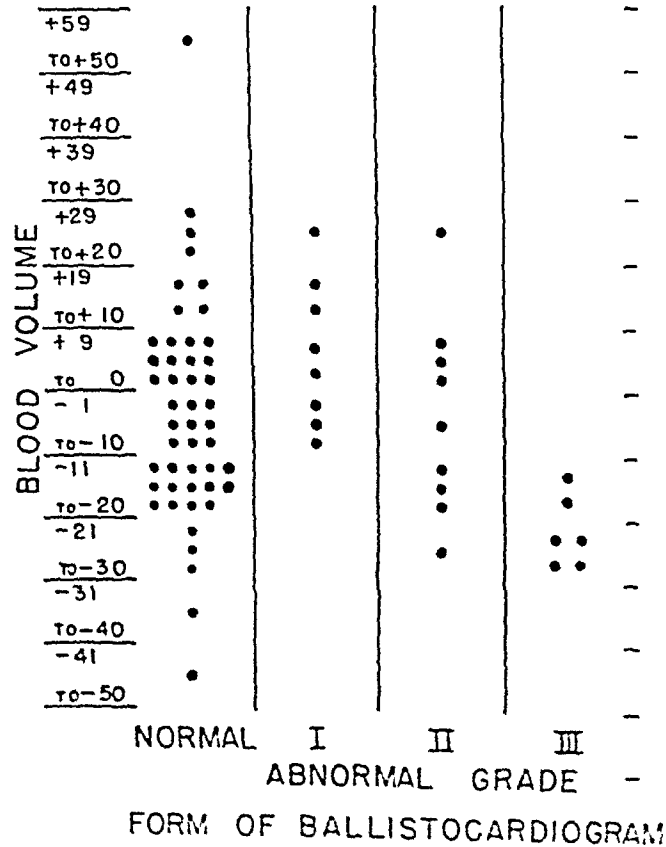


FIG. 6.—Relation of blood volume to the degree of abnormality of the form of ballistocardiograms.

plasma volume and also the hematocrit with certain aspects of the circulation mentioned above. The relation between plasma volume and the frequency of abnormal ballistic forms just missed significance.

Discussion. The main purpose of the investigation was to discover whether patients whose stores of nitrogen were maintained by special methods of feeding before or after operation were in reality in better condition than those who went into more negative nitrogen balance. The surgeons in charge obtained the clinical impression that the patients given extra feedings were benefited. We sought to confirm this impression by objective evidence, and we believe that we have done so.

Postoperative abnormalities of form of the ballistocardiogram occurred far more frequently in those in negative than in those in positive nitrogen balance, and this difference is significant. However, such factors, as age and type of operation, might have played a part in this result. When the data were arranged to eliminate these factors, we failed to demonstrate statistical significance, but the odds are still about 10 to 1 that the patients in better nitrogen balance were more normal. We doubt if many well-accepted clinical notions are better based.

On the average the patients in better nitrogen balance stayed in the hospital a shorter time, and all survived to leave the hospital. Two of those in poorer metabolic condition died. Again neither of these differences is statistically significant, but added to the results mentioned, the evidence grows stronger. All our data support the clinical impression that those maintained in better metabolic condition were in better physical condition as well.

In addition to this demonstration we have drawn a picture in Table 4 of the average condition of the circulation of patients after severe operation. Our principal finding, a rise of pulse rate after operation, is an old story to anyone accustomed to looking at the charts in a surgical

ward. It may be of some value, however, to give the statistics of this well-known phenomenon. Otherwise there were few changes of importance.

These results differ somewhat from those we obtained after operations for hernia as the cardiac output was found to be depressed after operation in that group. The most probable reason for the difference was mentioned in our previous paper. Intravenous fluids were given in large amounts to the seriously ill patients in this study while they were rarely administered to the healthy persons subjected to herniorrhaphy.¹³ Intravenous fluids have been shown to support the circulation in the sick.⁵ The modern surgical routine of giving fluids intravenously during and after severe operations is upheld by our results, and the amount given to our patients can now be judged as satisfactory.

Finally we have some interesting data on the relation of blood volume to the circulation, for there is positive correlation between blood volume and cardiac output per minute. This was found when the whole group was studied, and it is consistent with the view that the filling of the heart was improved by the increased amount of blood, as found in the closed circuit heart-lung preparation. When changes occurring in single patients are studied, this relationship is not always apparent.

Equally interesting is the relation between blood volume and abnormal ballistic form, for the most abnormal types of ballistocardiograms occur most frequently in patients with unusually small blood volumes. One wonders whether poor cardiac filling was not a factor in the production of this abnormality. That poor cardiac filling does cause abnormalities is shown by the fact that in many records obtained in persons with obvious heart disease abnormalities of form occur only in that part of the respiratory cycle where cardiac filling is poorest. In a few instances abnormalities of form have been produced in healthy persons by a maneuver¹⁵ which suddenly reduced cardiac filling.

The elusive relation between disease of the gall bladder and heart disease has again appeared in this study. In the 10 cases of gall bladder disease proved at operation, 6 had cardiac outputs which were subnormal before they were operated upon when judged by our old normal standards. Indeed, the average of the entire group is subnormal, -26% below average normal. The presence of obesity will account for this abnormal result in only 1 case. In addition, there was a strong tendency for these patients to develop ballistocardiograms with an abnormal form after operation (Table 1).

Summary and Conclusions. Ballistocardiograms and estimations of pulse rate and blood pressure were made on 63 patients before and frequently after serious surgical procedures. Studies of nitrogen balance were made in 36 of these patients, and blood volume was estimated in 34.

The changes in the circulation occurring during convalescence from severe operations have been studied statistically.

Patients kept in nitrogen balance by extra feeding exhibited less abnormality of the circulation after operation than those who went into negative nitrogen balance. This objective finding agreed with the clinical impression that the cases kept in nitrogen balance stood their operations better.

There was significant correlation between the blood volume and cardiac output when the group was considered as a whole. Extremely abnormal ballistocardiograms occurred chiefly when the blood volume was small.

Our cases of biliary tract disease showed a pronounced tendency to have subnormal circulations before operation and to develop abnormal ballistocardiograms after operation.

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POSTURAL HEART BLOCK

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THE amount of literature pertaining to heart block in relation to varying positions of the body is not outstanding. Several treatises have recently appeared which discussed this subject, illustrated with cases of first or second degree heart block. A careful survey of the available medical journals failed to reveal any instance involving a third degree or complete heart block.

We have recently cared for 2 patients, 1 of whom demonstrated complete heart block while recumbent. In the sitting position this was converted to a first degree type. The other patient showed heart block secondary to digitalis medication. While supine, dropped beats (second degree heart block) were present but disappeared when the patient assumed the upright posture.

The first case is reported because of its rarity. Both patients present features illustrating the vagal character of some types of heart block.

Case Reports. A Mexican, aged 19, felt perfectly well until 1943, when he began to have vague abdominal pain and lost much weight. He vomited frequently, but his bowels were regular. For the first time on Jan. 1, 1944, and then subsequently at irregular occasions, he had the sensation of "being paralyzed," his wrists and legs seemed to be numb and both his hands contracted. These episodes lasted about 20 minutes and disappeared spontaneously. He had been short of breath on moderate effort and occasionally had a "suffocating" feeling at night. There had never been any edema nor any orthopnea; occasional nocturia was noted. No history of rheumatic fever, diphtheria or hypertension was obtained. As a child he had scarlet fever.

Examination on admission revealed an apprehensive patient not acutely ill. The findings were limited to the heart. The heart was not enlarged. Sounds were of fair quality, P2 greater than A2. There

was a short, soft systolic murmur heard at the pulmonic area. The rhythm was regular. In the sitting position, the rate was 90 per minute; lying down the rate dropped to about 45 per minute. Electrocardiographic tracings taken in both these positions revealed that in the sitting position he had a first degree heart block (prolonged PR interval) while in the recumbent position he had a complete heart block (Fig. 1). This patient was observed during several of his "attacks." At those times he was quite excited, but hyperventilation was not evident. The lips were slightly cyanotic. Pulse was of fair quality and rate about 105 per minute. Carpopedal spasm was present. One of these attacks was relieved by adrenalin subcutaneously, and another stopped spontaneously before any medication could be given to him. Atropine, $\frac{1}{2}$ gr., administered subcutaneously during one of the periods of complete block changed it to a first degree type (Fig. 2). Subsequently the complete heart block disappeared but a prolonged PR interval as long as 0.44 second persisted throughout his 4 months hospitalization. Fluoroscopic examination failed to reveal any cardiac chamber enlargement.

Because of the patient's complaints that he had lots of "gas," a gall bladder series, gastro-intestinal studies and gastric analysis were performed, all of which were within normal limits. Attempts at hyperventilation failed to reproduce patient's original symptoms. Sedimentation rate determinations were always within normal limit. His temperature was normal. Blood sugar was 104 mg. per 100 cc., blood calcium 10.7 mg. per 100 cc., blood phosphorus 3 mg. per 100 cc. During one "attack" the blood phosphorus was 5.6 mg. and blood calcium 8.4 mg. Stool examinations were negative.

The patient became asymptomatic on a moderately restricted physical program, but on discharge the first degree heart block persisted. It might be noted that during the changing block there were never any symptoms suggestive of the Stokes-Adams syndrome.

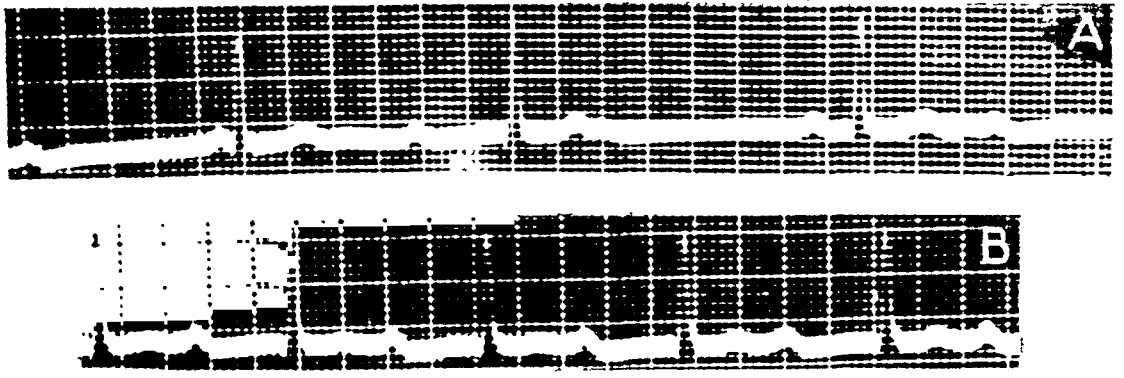


FIG. 1. Case 1.—*A*, Recumbent position; complete heart block. *B*, Sitting position, first degree heart block (PR interval = 0.44 second.)

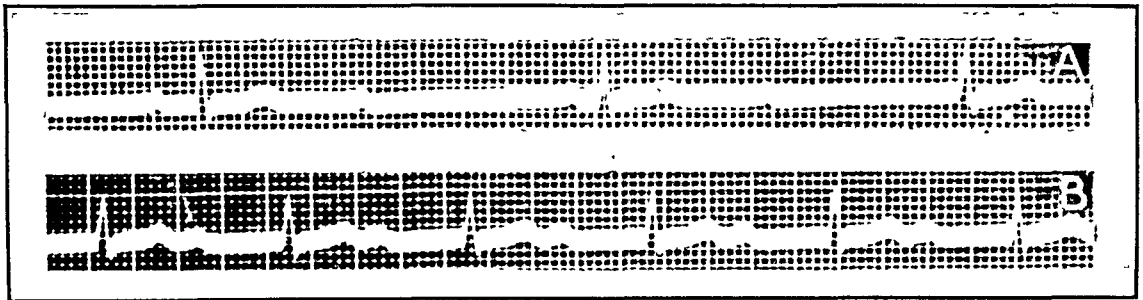


FIG. 2. Case 1.—*A*, Recumbent position; complete heart block. *B*, Recumbent position; first degree heart block (PR interval = 0.14 second); after atropine gr. $\frac{1}{16}$ subcutaneously.

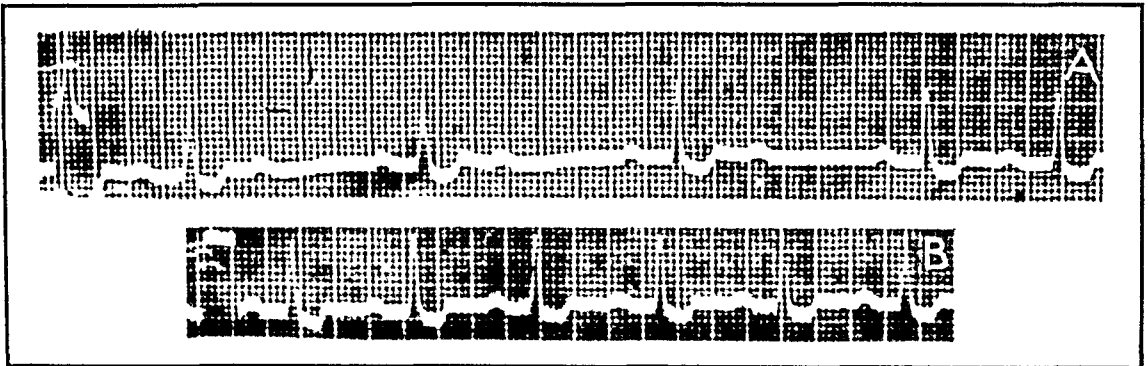


FIG. 3. Case 2.—*A*, Recumbent position; second degree heart block, digitalis effect. *B*, Sitting position; first degree heart block (PR interval = 0.24 second.)

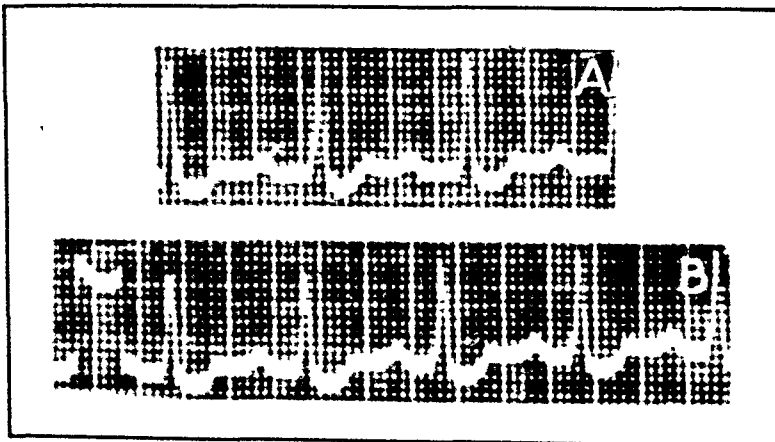


FIG. 4. Case 2.—*A*, Recumbent position; first degree heart block (PR interval = 0.26 second). *B*, Recumbent position; following administration of full dose of atropine = PR 0.20 second.

STEIN: POSTURAL HEART BLOCK

CASE 2. A 50 year old white male was first observed in October 1945 because of shortness of breath on effort, nocturnal dyspnea, and pain in the right upper quadrant, which he had had for a period of 1 month before admission. Physical examination revealed a chronically ill patient, looking older than his chronologic age, with a few fine râles at both bases, a gallop rhythm at the apex, an enlarged tender liver, and 1+ bilateral pitting pretibial edema. The electrocardiogram was abnormal. He was put to bed and digitalis therapy instituted with very prompt improvement in his condition. He was discharged after 2 weeks and he continued on a maintenance dose of digitalis as an out-patient.

Subsequent examination on Dec. 6, 1945, revealed an irregular cardiac rhythm in that a beat was lost at irregular intervals. This was present while in the recumbent position. However, when sitting up the rate was more rapid and regular. There were no signs of decompensation. Electrocardiographic studies revealed a prolongation of the PR interval, ST segment depression and in the recumbent position, irregularly dropped beats (second degree heart block). In the sitting position the second degree heart block disappeared but the prolonged duration of the PR interval persisted (Fig. 3). The administration of full doses of atropine reduced the PR interval from 0.26 to 0.2 second (Fig. 4). These abnormal findings were due to digitalis. When the drug was discontinued the block disappeared. However, there were never any symptoms of overdigitalization noted.

Discussion. Auriculo-ventricular heart block has customarily been viewed as being either on an organic or functional basis. If exercise or full doses of atropine caused the disappearance of the block, it was taken as evidence of vagotonia, and thus functional in character. Should the prolonged PR interval persist despite these measures the process was deemed to have an underlying organic basis. The latter were thought to be usually due to rheumatic fever, particularly in the younger age group, and to arteriosclerosis in the older. It has been conclusively demonstrated, however, that a definite vagal element exists in the A-V block of

early rheumatic fever as suggested by Bruenn² and others.⁸

On the other hand it cannot always be assumed that when atropine medication reverts the PR interval to normal, there does not exist a lesion at the myoneural junction, in the conduction system or in the heart muscle which is responsible for the block. Carter and Dieuaide³ described a case of recurrent complete heart block in which the PR interval returned to normal with atropine, yet necropsy revealed a badly diseased bundle.

The relation of heart block to changes in posture has received insufficient stress in the medical literature. Holmes and Weill⁴ suggest that all cases of incomplete heart block be studied for postural evaluation. These writers presented 2 cases showing first and second degree heart block in the supine position. In both of these cases the block was abolished when the subjects assumed the upright posture.

Previously Alexander and Bauerlein¹ noted a patient in whom heart block was observed in the supine position and disappeared when standing. Clinostatic heart block was also observed by Poel⁵ who presented a case in which a change in posture from lying to standing changed the PR duration. He attributed this to vagal influence. Manning and Stewart⁶ during routine electrocardiographic studies of Royal Canadian Air Force crew discovered 4 cases which showed significant heart block in the recumbent position, which disappeared when upright. In 1, the PR interval was 0.4 second in the supine position and 0.2 second when upright. They assumed that it was a vagal phenomenon. Two unusual cases were studied by Miller and Perelman⁷ in which clinostatic incomplete A-V block was found accompanying auricular tachycardia. They explained the first on the basis of fatigue consequent upon the rapid rate and enhanced vagal tonicity.

In both of our patients the evidence pointed toward vagal hyperactivity as underlying the heart block. Case 1 was

an individual emotionally unstable and with unmistakable evidence of autonomic nervous system imbalance. In Case 2 as mentioned previously the prolongation of the PR interval and the dropped beats were produced by digitalis. According to White⁹ digitalis affects A-V conduction, at least in part, by vagal stimulation. In our patient it may have been the entire story as the PR interval was reduced from 0.26 to 0.2 second (Fig. 4) following atropinization. It is to be noted that in Case 1 the auricular rate increased when the patient assumed the sitting position, while Case 2 showed no rise in rate in the upright position.

It would therefore seem warranted in all instances of prolonged A-V conduction time to note any deviations produced by postural changes. The factors responsible

are probably on the basis of a hypertonic state of the vagus nerves.

Summary and Conclusions. 1. Two cases of postural deviations in the A-V conduction time are described. In the 1st instance complete heart block was present in the recumbent attitude and lesser degrees of block when upright.

2. The 2nd patient presented a second degree heart block while supine and a first degree in the standing position. The block was caused by digitalis.

3. Because both showed reduction of the block in response to atropine, vagal action was assumed as responsible for the above findings.

4. All patients with A-V conduction disturbances should be studied in several postures for more complete evaluation of the clinical and electrocardiographic pictures.

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PULMONARY INFILTRATIONS WITH ASSOCIATED EOSINOPHILIA

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AND

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IN 1932 Löffler described the syndrome of transitory pulmonary infiltration associated with eosinophilia.¹¹ By 1936 he had accumulated 51 cases which he reported.¹² The main characteristic of these infiltrates was their sudden appearance and disappearance in 3 to 8 days. The associated eosinophilia averaged 10 to 30%, but in some instances was over 60%. Cases have been subsequently reported, however, in which the symptoms were more severe and the clinical course more chronic. The pulmonary infiltrations associated with these cases could be demonstrated roentgenologically for weeks or months.^{6,7,8,18}

Löffler first thought the condition might be a benign form of tuberculosis. He later discarded this theory in favor of one that the eosinophilia was the expression of an anaphylactic process. Engel of Shanghai^{4,5} was the first to provide substantial evidence that pulmonary infiltrations associated with eosinophilia may occur on an allergic basis. A species of *ligustrum*, called privet, blooms in China during May and June. At this time large numbers of people have a type of bronchitis, commonly referred to as "privet cough." Roentgenographically there is pulmonary infiltration, lasting 1 to 6 days, associated with eosinophilia of 20 to 25%. Maier, after reviewing 100 cases of transitory pulmonary infiltrations with blood eosinophilia, also came to the conclusion that they are allergic in nature.¹³ On the other hand, pulmonary infiltrations with eosinophilia have also been described in association with trichinosis,^{14,15} chronic brucellosis,³ and amebiasis,⁹ and infestation with necator,¹⁵ *Fasciola hepatica*,¹⁶

Ascaris lumbricoides,¹⁹ and *Strongyloides stercoralis*.¹ The differential diagnosis of pulmonary infiltrations with eosinophilia therefore entails the consideration of many factors. It is the purpose of this communication to emphasize another condition in which pulmonary infiltration may be associated with moderate or marked eosinophilia. The occurrence of eosinophilia in coccidioidomycosis has been reported by others.^{2,17,20} In the literature on the subject available to us, however, the eosinophilia has been slight, ranging from 5 to 18%. In a previous communication,²⁰ one of us reported a case of pulmonary coccidioidomycosis with 31% eosinophilia. We now wish to report 2 additional cases, 1 with 89% eosinophilia, and another with 27%.

Case Reports. CASE 1. A 27 year old radio operator of Mexican extraction was admitted to the March Field Station Hospital on Oct. 22, 1944, with a chief complaint of chills and fever of 36 hours duration. On October 19 the patient was flying a night mission. About 1 o'clock, October 20, he began having chills and fever associated with some headache and aching of the muscles, especially in the back. He had chills all that day and the next. On October 22 he reported to sick call and was admitted to the hospital. There were no symptoms other than those stated above.

In civilian life, the patient was a clerk working in an office in Los Angeles. There was no history of tuberculosis or allergy. In 1942 he was operated on for what he stated was a "ruptured stomach ulcer."

The patient had been in the Army since Oct. 2, 1943. During that time he was at Air Bases in Denver, Colo.; Sioux Falls, S. D.; and Yuma, Ariz. He was at Los Angeles

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Army Air Field, Lemoore, Calif., a known area of endemicity, from Sept. 22, 1944, to Oct. 9, 1944. He arrived at March Field Oct. 10, 1944.

On admission to the hospital, patient's temperature was 102° F. Physical examination was essentially negative. The chest was clear. There was a well-healed right pararectus scar from his previous operation. White blood cell count was 19,900 (57% neutrophils, 25% lymphocytes, 17% eosinophils and 1% basophils). Red blood count was 4,950,000 with 15 gm. hemoglobin. Roentgenogram of the chest, blood Kahn and urinalysis were negative.

The patient was started on 1 gm. sulfadiazine orally every 4 hours. After 48 hours his temperature fell to normal and remained so for 2½ days, at which time it rose to 99.4° F. At this time, physical examination was again negative. A roentgenogram of the chest, taken Oct. 27, 1944, showed a diffuse granular density in the second, fourth and fifth left interspaces anteriorly. On October 29 the patient had 3 isolated pustular lesions on his back, but otherwise looked and felt well. The initial symptoms had disappeared, and his temperature was normal. White blood count at this time was 32,100 (67% eosinophils, 19% lymphocytes and 14% neutrophils). Sulfadiazine was discontinued and a search made for the cause of the marked eosinophilia. Eight stool examinations were negative for ova, parasites and post-culture. Two blood agglutinations for brucellosis were negative in all dilutions. Trichinella skin test was negative. Several sputum examinations were negative for significant findings. Tuberculin (Purified Protein Derivative) was negative in the first strength but 4+ in 48 hours in the second strength. Coccidioidin skin test (1:100) was 2+ positive in 24 hours and 48 hours.

A tentative clinical diagnosis of coccidioidomycosis was established, with eosinophilic leukemia and parasitism to be ruled out.

On November 5, after having had a normal temperature for 6 days, the patient had a fever of 100° F. He now had a pustular lesion on his right leg similar to those on his back. The posterior cervical and left axillary lymph nodes were enlarged, firm, slightly tender, but discrete and movable. The liver and spleen were not enlarged.

The next day the patient had a chill with a fever of 102.6° F. and again the following afternoon. At this time the white blood count was 45,700 (80% eosinophils, 6% neutrophils, 5% lymphocytes). Roentgenograms of the chest showed the previously described infiltration in the left chest. In addition, there were small areas of infiltration in the right fifth anterior interspace. On Nov. 8, 1944, the patient developed a pustular lesion on his scalp. Those present on his back were now dark red, firm and appeared to be infiltrating the surrounding skin.

To rule out the possibility of an eosinophilic leukemia, a sternal bone marrow aspiration was done. The white blood count at this time was 49,650 (80% eosinophils, 7% lymphocytes and 4% neutrophils). The bone marrow examination was done by Capt. D. T. Edmeades, Chief of Pathology Service, and was reported as follows: Differential bone marrow smear (600): eosinophil percentages: segmented 37, staff cells 31.2, metamyelocytes 5.3, myelocytes 3; neutrophil percentages: segmented 2.2, staff cells 3.6, metamyelocytes 2.5, myelocytes 0.3; premyelocytes 2, myeloblasts 0.2, eosinophilic normoblasts, 3.9, megaloblasts, 0.5, lymphocytes, 2.9, atypical lymphocytes, 1.1, undifferentiated small cells 1.6.

There is marked eosinophilic proliferation and infiltration of the bone marrow. Predominantly the cells are of the mature type with only 2.2% myeloblasts and premyelocytes. While the smear might be consistent with so-called chronic eosinophilic leukemia, these changes would also be seen in any disease producing a marked stimulation of eosinophils.

On Nov. 11, 1944, a biopsy of a lymph node and portion of a skin lesion was done. The pathologic report was as follows: lymph node: portions of the normal architecture are distorted due to the formation of pseudotubercles. Some of these show central caseation necrosis surrounded by epithelioid and reticulo-endothelial cells. In some of these areas there are spherical bodies measuring 15 to 60 micra which take the basic stain and are probably calcified. In other areas the center of the tubercle contains many polymorphonuclear cells forming a small abscess. In still others there are 1 to 3 giant cells usually of the Langhans type. A rare giant cell contains a spherical body with a

doubly refractile cyst wall and occasionally with endospores which are typical of *Coccidioides immitis*. There is an increase in fibrous tissue about the tubercles and marked reticulo-endothelial proliferation which in some areas resembles Boeck's sarcoid. Between aggregations of tubercles there is marked infiltration of polymorphonuclear cells and a few monocytes and eosinophils.

Skin: section is through skin with rather extensive micro-abscess formation in both the corium and the subcutaneous tissues. Surrounding these abscesses and extending along the sweat glands there is a neutrophilic, eosinophilic and lymphocytic infiltration. A rare spherical cyst containing endospores typical of *C. immitis* is seen. Deep in the infiltrate there is a rather marked hyperplasia of reticulo-endothelial cells. Superficially, 1 of the small abscesses has ruptured externally. The surrounding stratified squamous epithelium shows alternating areas of atrophy and hyperplastic rete pegs. There are accumulations of degenerating neutrophils between the stratum corneum and the granular layers. Rather marked parakeratosis is present.

Diagnosis: Coccidioidomycosis, disseminated, lymph node and skin.

Dr. C. E. Smith of Stanford University performed serologic examinations of the blood for coccidioidal infection. The report was as follows: Complement fixation tests: serial dilutions of serum (0.25 cc.): 1:2, +++++; 1:4, +++++; 1:8, +++++; 1:16, +++++; 1:32, +++++; 1:64, ++; 1:128, 0; 1:256, 0. Precipitin tests: serial dilutions of antigen: undiluted, ++; 1:10, ++; 1:40, ++; 1:100, 0.

Conclusion: Coccidioidal infection with dissemination.

The patient was started on a course of treatment using Ball Mill *Coccidioides* Vaccine.* At this time the white blood count

was 40,500 (76% eosinophils, 14% lymphocytes, 10% neutrophils). The sedimentation rate (Wintrobe) was 34 mm./hour.

The patient tolerated the vaccine well and showed definite clinical improvement. His temperature remained normal. Physical examination of chest remained negative. On Dec. 17, 1944, a roentgenogram of the chest showed a definite reduction in the amount of infiltration in both lungs. The white blood count at this time was 15,150 with a differential of 42% eosinophils, 30% lymphocytes, 26% neutrophils and 2% basophils. Sedimentation rate was 12 mm. hour.

By Jan. 2, 1945, all roentgenographic evidence of pulmonary infiltration had completely disappeared. The white blood count was 10,150 (38% eosinophils, 37% lymphocytes, 25% neutrophils). Sedimentation rate was 32 mm./hour. A re-check serologic examination showed the following: Complement fixation tests: 1:2, +++++; 1:4, +++++; 1:8, +++++; 1:16, +++++; 1:32, +++++; 1:64, +++++; 1:128, +++++; 1:256, +++++. Precipitin tests: undiluted, 0; 1:10, 0; 1:40, 0; 1:100, 0.

Conclusion: No significant change from previous examination.

On Jan. 19, 1945, the patient was transferred to another hospital for further convalescence. At that time his chest was clear roentgenographically; the white blood count was 9750 with 23% eosinophils, 35% lymphocytes, and 45% neutrophils. Sedimentation rate was 27 mm./hour.

The patient was next seen at the March Field Station Hospital on July 28. He felt well, had gained weight, but ran an occasional elevation of temperature to 99.6° F. at 4 p.m. Roentgenogram of the chest was negative. Examination of the blood showed a white blood count of 7250 (6% eosinophils, 45% lymphocytes, 48% neutrophils, 1% basophils). The red blood count was

* Supplied by Dr. Charles E. Smith, Stanford University, California. The material is a ball mill grind of coccidioides mat in a liquid synthetic media in which the fungus has grown for 2 months. It contains aqueous merthiolate 1:10,000 and is purified by Berkefeld filtration.

To use the vaccine, one first skin tests with 0.1 cc. of 1:10, 1:100 and 1:1000 dilutions. Then selecting the concentration which causes a moderate reaction (say 1 cm.), one injects that amount intramuscularly. Two days later, if the local and systemic reactions have not been severe, the vaccine is injected intravenously. The usual initial dose is 0.1 cc. of a 1:100 dilution. Every 2 days a dose, double the preceding one, is given. A slight fever or chill is no contraindication to further treatment. If a marked reaction occurs the dosage should not be increased but held at the present level. When one reaches 1 cc. of any dilution, the next dose should be 0.1 cc. of the next lower dilution. For example, 1 cc. of 1:100 dilution followed by 0.1 cc. of 1:10, 1 cc. of 1:10 followed by 0.1 cc. of undiluted vaccine. The dosage is not increased beyond 2 cc. of undiluted material. A course at 15 to 20 cc. of vaccine may be given twice weekly for 6 weeks. If further vaccine therapy is indicated the whole course is repeated.

4,710,000 with 15 gm. hemoglobin. The sedimentation rate was 20 mm./hour. A specimen was taken for serologic examination. The report was as follows: Complement fixation tests: 1:2, +++++; 1:4, +++++; 1:8, +++++; 1:16, +++++; 1:32, +++++; 1:64, +; 1:128, 0; 1:256, 0. Precipitin tests: undiluted, 0; 1:10, 0; 1:40, 0; 1:100, 0.

Further follow-up examination on this patient has not been possible.

CASE 2. A 28 year old white male bombardier of Mexican extraction was admitted to the March Field Station Hospital on Aug. 20, 1945, with the complaint of fever and malaise of a few hours duration. He had noticed symptoms of a mild upper respiratory infection for the past 2 weeks, manifested by nasal congestion, slight sore throat, mild unproductive cough and anorexia. In the 12 hours preceding hospital admission he had noticed fever, weakness and slight substernal pain on coughing.

On admission, he was found to have a temperature of 101° F., pulse 94 per minute and blood pressure was 125/75. There was a moderately injected pharynx; heart and lungs were clear on examination. The remainder of the physical examination was negative. Complete blood count: red blood cells, 4,800,000; hemoglobin, 14.2 gm.; white blood cells, 12,500 (75% neutrophils, 25% lymphocytes); Kahn negative; throat culture, a light growth of alpha hemolytic streptococci; direct smear of the throat, a few gram-positive cocci in pairs.

Roentgenogram of the chest taken Aug. 21, 1945, was essentially negative. The clinical impression at that time was nasopharyngitis.

His course was one of continued fever, and an annoying hacking unproductive cough. The physical examination remained essentially negative.

On August 27, roentgenogram of the chest was reported as showing "rather heavy hilar markings and a probable increase in the size of the hilar nodes on the left. No definite parenchymal infiltration made out." The white blood cell count on this date also showed the total cell count to be 16,000 (57% neutrophils, 21% lymphocytes and 22% eosinophils). The sedimentation rate was 26 mm. hour (Wintrobe) corrected. Several sputum examinations were found negative for significant organisms. A number of blood agglutination tests were per-

formed, including Widal and heterophil agglutinations without significant findings. A blood culture was negative. Repeated stool examinations for ova and parasites as well as cultures were negative. Agglutinations for brucella were negative.

On August 27 a skin test was done using coccidioidin in a dilution of 1:100. This was found to be positive in the course of 36 hours. Sputum examinations, again made, were also found negative. Clinical impression at this time was pulmonary coccidioidomycosis.

On September 8 a roentgenogram of the chest was reported as showing "parenchymal infiltration in the left lung field at the level of the fourth and fifth intercostal spaces anteriorly, along with prominence of the mediastinal nodes on the left." The sedimentation rate on this date was 32 mm./hour (Wintrobe) corrected. White blood cell count was 14,000 (59% neutrophils, 14% lymphocytes and 27% eosinophils). The patient continued to feel weak, had no appetite and ran a temperature from 100° F. to 101° F. Physical examination remained negative.

Serologic examination of blood taken Sept. 10, 1945 (performed by Dr. C. E. Smith of Stanford University Medical School) was reported as follows: 1:2, +++++; 1:4, +++++; 1:8, +; 1:16, 0; 1:32, 0; 1:64, 0; 1:128, 0; 1:256, 0. Precipitin tests: serial dilution of serum (0 to 25 cc.): undiluted, +++++; 1:10, +++++; 1:40, +; 1:100, 0.

Conclusion: Findings indicated a primary coccidioidal infection, well focalized.

On September 15 the patient became afebrile, showed very marked subjective improvement with increase in appetite, and a feeling of wellness. On this date the white blood cell count was 12,000 with 60% neutrophils, 18% lymphocytes and 22% eosinophils. The sedimentation rate was 32 mm./hour (Wintrobe) corrected.

On September 24 the chest roentgenogram showed definite regression and improvement of the parenchymal infiltration, and diminution in the size of the hilar nodes.

The remainder of the clinical course was uneventful. A chest roentgenogram on Oct. 12, 1945, was reported essentially negative. On this date, also, the white blood cell count was 10,600 with 74% neutrophils, 24% lymphocytes and 2% eosinophils. The sedimentation rate continued to decline, and

the patient was returned to duty after a total of 65 days of hospitalization.

Discussion. In the 2 cases described, pulmonary infiltration lasted approximately 67 days (Case 1) and 46 days (Case 2) respectively. Blood eosinophilia, likewise persisted for the same approximate duration. We and others have seen cases in which the pulmonary infiltrations were of shorter duration, and in which the eosinophilia was less pronounced.¹⁷ Case 1 is interesting because of the unusually high eosinophilia, and the seemingly fair chance of recovery from the disseminated form of coccidioidomycosis.

It may be assumed that a considerable number of transient persons have either

recently resided in or traveled through endemic areas for *Coccidioides immitis*. These persons could easily arrive at their destinations in non-endemic areas, and there develop the disease within the accepted incubation time. It is also conceivable that some perplexity might arise concerning the diagnosis in those areas where the disease is seen infrequently. It is suggested, therefore, that the differential diagnosis of pulmonary infiltrations with eosinophilia should include the possibility of coccidioidomycosis.

Summary. Two cases of coccidioidomycosis are reported. Both cases demonstrated pulmonary infiltration with high blood eosinophil levels, 89 and 27 % respectively.

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DRUGS USED IN CLINICAL DIAGNOSIS (PART II) REVIEW OF RECENT LITERATURE

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IN the November 1944 issue of this Journal a review of recent literature on drugs used in clinical diagnosis was presented. The analysis covered in particular the more important American literature during the past 10 years, and related chiefly to drugs used in the diagnosis of liver function, namely, bromsulphalein, benzoic acid, galactose, bilirubin, tyrosine, amino acids, azorubin-S, vitamin K and dextrose. The analysis of the literature has now been extended to include gastro-intestinal function, pancreatic function, the gall bladder, epilepsy, myasthenia gravis and pregnancy. This review forms the subject of the present report.

GASTRO-INTESTINAL FUNCTION. *Neutral Red.* The dye, aminodimethyl tolu aminozone hydrochloride, was introduced as a test of gastric function in 1923 Glaessner and Wittgenstein⁴⁶). The test depends on the fact that some of this substance is excreted into the gastric contents after parenteral administration (Finkelstein,⁴⁷ Fuld⁴⁸), and in gastric disturbances, depending on the underlying disorder, its appearance is accelerated,

delayed, or absent (Glaessner⁴⁶). The basis of the method involves determining the time interval by frequent gastric aspirations. The method tests the excretory function of the parietal cells inasmuch as they selectively eliminate the dye (Morrison;⁴⁹ Morrison, Gardner and Reeves⁷⁸). This function is apparently distinct from that of acid secretion, as they frequently do not parallel each other either in the normal or abnormal stomach (Gillman,⁴⁵ Winkelstein¹²⁵).

In the original test a dose of 5 cc. of a 1% solution (50 mg.) was injected intramuscularly and gastric juice was collected every 2 minutes until the dye appeared. The normal standard was 15 to 20 minutes. Its appearance sooner than 10 minutes or later than 20 minutes was taken as abnormal.^{46,47} Several modifications have been introduced aimed at increasing the sensitiveness of the test. Various plans for taking gastric samples at intervals ranging from $\frac{1}{2}$ to 5 minutes have been suggested (Gillman,⁴⁵ Ollerens,⁴⁴ Robertson⁵⁰) and administration of the drug has been changed to the intravenous

route.^{45,95} The fact that the gastric mucosa, beside excreting this compound, concentrates it to a considerable degree, suggested still another modification;⁴⁵ the test dose (5 cc. of a 1% solution) was given intravenously and samples of gastric contents were taken at minute-intervals for the first 30 minutes. The degree of maximum concentration and the time taken to reach it, *i. e.*, the concentration time, were determined in addition to the excretion time, *i. e.*, time it takes for dye to appear following the injection. If dye excretion was abnormal at the end of 30 minutes, 0.5 mg. of histamine acid phosphate was given subcutaneously and the test continued for a further 90 minute period. In individuals free of gastric dysfunction the average excretion time was 4 minutes and the average concentration time was 14 minutes. The upper limits of normal were taken as 6⁹⁵ or 9⁴⁵ minutes for the former and 20 minutes^{45,93} for the latter. Patients with chronic gastritis, gastric carcinoma and chronic disease associated with gastric dysfunction, *e. g.*, pernicious anemia, nephritis, liver disease, or severe clinical avitaminosis, displayed an absence or impairment of dye excretion and/or concentration.^{45,93} In no case where there was an absence of dye excretion was an acid secreting response to histamine observed. In contrast, patients with gastric or duodenal ulcer showed an acceleration of these functions and in some individuals the resultant excretion and concentration times were as brief as $\frac{1}{2}$ and 2 minutes respectively.⁹³

A combined test (neutral red, histamine and a test meal) was employed in individuals exhibiting a 2 hour achlorhydria by the routine Rehfuess fractional method (Winkelstein¹²³). In 15 cases the dye appeared in the absence of free HCl. Each sample was acidified to bring out the red color of the dye. Since, in all instances, free HCl was subsequently demonstrated by more intensive methods (additional histamine, alcohol, oranges, etc.), the dye test appears to be more sensitive than histamine as an indicator of parietal cell

function. In addition, when repeated in the same individual on different occasions, relatively constant results were obtained while histamine produced wide variations.⁴⁵ The combined test is apparently more sensitive in the diagnosis of true achlorhydria than any previously employed.¹²⁵ Fourteen patients exhibited achlorhydria of unknown etiology in a study with this test. On gastroscopic examination all were found to have gastritis of either the atrophic, hypertrophic or mixed hypertrophic form. In an earlier gastroscopic study of 161 patients tested with neutral red it was noted that severe atrophic gastritis considered typical of pernicious anemia was associated with failure of dye excretion (Henning and Jürgens⁵⁷). Those with histamine-resistant achlorhydria, but with less severe atrophic lesions, eliminated the dye.

The fact that neutral red is excreted in tropical sprue with histamine-resistant achlorhydria, but not in pernicious anemia, has been applied in the differential diagnosis of these conditions (Olleros⁶¹). On occasion it has been found in the stomach contents of pernicious anemia patients (Cohen, Matzner and Gray²¹). In these cases it is the result of duodenal regurgitation of dye, for it has been shown to be excreted into the bile and duodenal secretion and by the entire small bowel, as well as in the gastric secretion (Pierce, Bockus and Bank⁴⁴). This difficulty can be overcome with the use of a double gastroduodenal tube and continuous suction (Bockus¹⁹). Beside its diagnostic value it has been suggested that the test might be used to gauge the progress of a gastric lesion or its recovery under therapy (Lourja⁷¹). This proposal has received little further evaluation since the Roentgen ray with an opaque medium serves the purpose so well. The test derives its greatest value from the fact that complete inability to excrete the dye is always indicative of gastric non-function, and that total failure of dye excretion is usually preceded by readily recognizable disturbances in dye excretion.

Histamine. The use of histamine (beta-imidazolylethylamine) for testing gastric function is based on the fact that it is a maximal stimulant to the secretion of hydrochloric acid. Its application has made possible the study of the secretory response of the gastric glands uninfluenced by psychic factors. It has also made available for study pure gastric juice uncontaminated by a test meal which, however simple, produced physical and chemical alterations through dilution and neutralization (Babkin⁸). The basis of the procedure involves performing gastric aspirations on a 12 hour fasted individual at 10 or 15 minute intervals for 1 hour following the hypodermic injection of a standard dose (0.1 mg. per 10 kg. of body weight) of the drug. The volume and acidity of the samples are determined. The normal response is prompt with maximum acidity being attained in 20 to 30 minutes. The procedure tests the acid secreting function of the parietal cells by direct stimulation. Atropine produces little or no alteration in response (Gray⁵⁰). There is apparently some lack of agreement as to whether it is also a stimulant to pepsin formation by the chief cells. There is 1 report which indicates that the secretory response to a second injection of histamine given 60 to 90 minutes after the first is similar to that following the first and exhibits chief cell as well as parietal cell activity (Rivers, Osterberg and Vanzant⁹⁷). This test, using a second injection, does not determine, however, whether histamine stimulates pepsin production or only mechanically promotes elimination of preformed pepsin (Bucher and Ivy¹⁹). The fact that the values for pepsin show no such correlation with histamine as do those for total chloride and HCl, and the not infrequent failure of pepsin concentration to rise above the fasting level after histamine, are further arguments against stimulation of the chief cell by this drug (Toby¹¹⁵). Most observers believe that histamine stimulates the parietal cell exclusively.^{4 115}

A modification of the histamine test

was explored in an attempt to secure information regarding the maximal potentiality of acid and pepsin secretion of the gastric cells and their ability to maintain increased secretory rates over longer periods of time. This double histamine test⁹⁷ is essentially a combination of the original 60 to 90 minute test, at the conclusion of which a second dose of the drug similar to the first is given, and specimens are again collected at 10 or 15 minute intervals. The normal individual shows a rise in acidity which falls within 60 to 90 minutes and rises again after the second injection. Patients with ulcer frequently exhibit a sustained high acidity curve throughout the entire test.

The fact that an alkaline tide occurs in the urine when hydrochloric acid secretion is taking place, and is absent in achlorhydria, has been made the basis of a gastric function test (Allen and Haft¹). The method involves injecting 0.3 mg. of histamine acid phosphate subcutaneously and giving 500 cc. of water by mouth after a fasting specimen of urine has been voided. Five hourly urine specimens are obtained and their pH determined. An alkaline tide occurred in those with normal gastric function while, with few exceptions (severe renal damage, urinary infections, etc.), it was absent in cases of achlorhydria.

The histamine test of gastric function has important limitations. The motor function of the stomach is not tested. It fails to give information concerning mucous and pepsin secretion,⁸ and the range of normal acidity is so great that it becomes difficult to decide what value shall be taken to mean normal, hyper- or hypoacidity (Ruffin and Dick¹⁰²). There are variations with sex in normal¹⁰² and ulcer individuals (Brown and Rivers¹⁵), and with age,¹⁰² the mean values being lower in females and in the elderly. While significant variations from control values occur in gastric carcinoma and pellagra, the more important information relates to the presence or absence of HCl, rather than the amount that is present.

In a recent study (Glenn⁴⁸) the test was applied in an attempt to differentiate duodenal ulcer from other conditions causing digestive disorders, *i. e.*, psychoneurosis, gastritis, etc. Attention was directed to both total acidity and total volume of gastric secretion. While differences for these values in the 2 groups occurred, the overlapping in distribution made the procedure one of little value in the diagnosis of ulcer. The main reason for using histamine remains the demonstration of achlorhydria.¹⁰² However, spontaneous variations in response to the drug do occur (Palmer, Kirsner and Nutter⁵⁶) and a "histamine-proved" achlorhydria is not necessarily indicative of permanent anacidity (Schiff, 1938).

Insulin. This substance, like histamine, is a stimulant to gastric secretory activity. However, its injection in the necessary dosage is followed by a strikingly different qualitative response. This fact has made it valuable as a diagnostic agent in the study of gastric function. The acidity of insulin stimulated gastric juice is only slightly lower than that obtained with histamine, and the total chloride concentrations are nearly equal. In addition, the juice obtained with insulin stimulation has large amounts of mucous and possesses considerable peptic activity.⁸ It becomes apparent, therefore, that insulin stimulates not only the parietal cells, but the other secretory elements of the gastric mucosa as well, *i. e.*, the peptic cells, the chief cells and the surface epithelium cells.⁸ The mechanism of its action, unlike histamine, is indirect. The hypoglycemia produced by insulin in the necessary dose stimulates the vagal gastric secretory centers in the brain. From this point impulses are transmitted down the gastric secretory fibers of the vagus nerves to the gastric glands.⁸ The secretory response can be abolished by raising the blood sugar to normal levels with intravenous glucose. The administration of atropine or section of the vagus nerve supply to the stomach will likewise prevent the response.⁸ This latter observa-

tion suggested that insulin hypoglycemia might be an excellent test for continuity of the vagus nerves (Babkin,⁹ Hollander⁴²). Recently, with the renewed interest in the supradiaphragmatic section of the vagus nerves for the treatment of gastric and duodenal ulcer, the test has been employed to determine if vagotomy was complete. The absence of a secretory response was found to be good evidence that the nerves had been completely sectioned (Thornton, Storer and Dragstedt;¹¹⁷ Dragstedt *et al.*⁷³). An acid gastric juice response indicated that some fibers had been left intact.⁴³

Insulin stimulation gives information concerning the first, or vagal, phase of gastric secretion. A study of this vagal secretory mechanism is of value when considering surgery of the stomach or duodenum for peptic ulcer (Babkin⁸). In a recent study (Welin and Frisk¹²¹) the use of the double gastroduodenal tube with continuous suction of duodenal contents through 1 lumen and gastric content through the other provided practically pure gastric juice. The samples were fractionated in 15 minute periods and, after 1 or 2 foreperiods, 20 units of insulin were injected intravenously and the test continued for 2 hours. In normal subjects there occurred a sudden and considerable increase in secretion within approximately $\frac{1}{2}$ hour following the injection. In those with low secretion rates it was delayed to 45 to 60 minutes. This augmented secretion was absent in achylia. The normal standard was taken as the secretion of 100 to 200 cc. in 60 minutes from the onset of response. Figures above and below these limits represented hyper- and hyposecretion respectively. Here, as after histamine, there is an overlapping distribution of values between normal and ulcer.

Caffeine. This xanthine is a stimulant to gastric secretion in man when given by mouth or injected intravenously (Babkin⁸ and Ivy¹¹⁸). The gastric juice obtained in response to its administration is qualitatively similar to that following histamine (Hart⁷²). Atropine does not prevent

the acid response. The effect of supra-diaphragmatic vagal section upon the gastric secretory response to caffeine was studied in individuals undergoing this treatment for peptic ulcer. It was found that the response was as clearly defined after the operation as before and in general was less than that produced by histamine (Thornton, Storer and Dragstedt¹¹⁷).

The secretagogue response to a "caffeine test meal" was compared in normal subjects and in "peptic" ulcer patients (Roth, Ivy and Atkinson¹⁰¹). In this procedure the stomach is emptied of residuum and, after a 30 minute "basal" control period, the test meal (0.5 gm. of caffeine with sodium benzoate in 200 cc. of warm water) is introduced through the tube. The stomach is emptied 30 minutes later and at 10 minute intervals thereafter for an additional 90 minutes. In normal and asymptomatic individuals, with the exception of those apparently having an ulcer predisposition, the total acidity output (volume times free and total acid concentration) returned to the control level within 60 to 80 minutes. Of 36 patients with "peptic" ulcer all but 1 consistently showed a prolonged secretory response. This type of curve was considered to be of diagnostic value inasmuch as there was little overlap in the response of normal and ulcer individuals, of the kind seen with alcohol and histamine. The mechanism of the prolonged secretory response was considered to be due to the synergistic action of caffeine with histamine which is thought to be liberated by patients with peptic ulcer. In a later study employing the same technique with minor modifications, the prolonged response was found in only 44% of 96 proved ulcer cases (Raimondi⁹³). Further study of the response in ulcer patients, noting especially the effect of remissions, is necessary before the test can be granted definite diagnostic value.

Priscol. Priscol, the proprietary name for 2-benzyl-4,5-imidazoline hydrochloride, was introduced as a stimulant to gastric secretion in 1940 (Thiele;¹¹³ Stalder¹¹²). It

apparently acts on the parietal cell since it evokes a response qualitatively similar to that of histamine. It is administered orally (50 to 100 cc. of a 1 to 2% solution) or subcutaneously (10 mg.). It is more potent subcutaneously (Nasio⁸⁰) and its quantitative action is superior to caffeine and slightly less than that of histamine. It apparently causes none of the side reactions occasionally seen with histamine. However, its failure to bring about maximum hydrochloric acid secretion makes it unreliable in the differential diagnosis of the achlorhydrias.

Phenolphthalein. The use of this drug in a test to determine the presence of gastro-intestinal lesions was introduced in 1938 by Woldman.¹²⁷ The method is based on the principle that a non-toxic substance which is not normally absorbed by the gastro-intestinal tract might, through any break in the mucosa, enter the circulation and be excreted in the urine.¹²⁷ Phenolphthalein was selected since it had been reported that after a small dose of the drug to normal individuals only the conjugated form (which does not yield a color reaction with alkali) was present in the urine. Free phenolphthalein was generally absent (Fantus and Dyniewicz³⁶). The finding of free phenolphthalein (which yields a pink color on the addition of alkali) in the urine was taken to indicate a break in the mucous membrane of the gastro-intestinal tract. The procedure involves collecting the urine obtained at 2 and 4 hour intervals after the administration of the test dose (10 cc. of a 1% alcoholic phenolphthalein solution) to a fasted individual and promptly testing it with 10% sodium hydroxide for free phenolphthalein. It was found positive in 34 of 35 patients with gastro-intestinal lesions and negative in 75 of 77 patients apparently free of such pathology. The test was thought to be valuable in differentiating between functional and organic disease, and in ruling out organic lesions of the gastro-intestinal tract in diseases producing digestive symptoms.¹²⁷ The reliability and value of the test were soon

questioned. The excretion of free phenolphthalein in the urine was apparently not related to breaks in the gastro-intestinal mucosa, but to the urinary concentration of the conjugated form. Whenever this concentration rose above 5 mg. % in any one specimen, free phenolphthalein appeared (Steigmann and Dyniewicz¹¹⁴). Other reports concerning the test's inaccuracy soon appeared. Many false positives occurred, particularly in individuals with blood dyscrasias or cardiovascular or infectious disease (Kremer, Shore and Wiesel⁶⁸). Its results were inconstant when repeated in the same individual (Notkin, Kirsch and Albert⁶²). It was ineffective in determining the presence or absence of tuberculous ulcers (Siltzbach and Nayer¹⁰⁹) and gave variable results in experimentally produced ulcers (Slutzky and Wilhelmj¹¹⁰). In 1 study (Le Vine and Kirsner⁷⁰) the "negative" error in those with gastro-intestinal disease was 38% while the "positive" error in those apparently free of such pathology was 46%. The test is obviously of no clinical value.

Hydrochloric Acid. The observation that the introduction of hydrochloric acid into the empty stomach of ulcer patients will produce the typical epigastric distress of an active lesion has been made the basis of a diagnostic test for this disorder (Palmer⁵⁵). The test is performed by emptying the stomach and introducing 200 cc. of 0.5% of the acid. If no pain occurs a second 200 cc. dose is given 30 minutes later and, if necessary, a third 200 cc. dose at the end of the hour. The patient is observed for 1½ hours and the test is considered negative if typical ulcer distress is not reproduced during this time. The test was usually positive in cases of gastric and duodenal ulcer and in certain cases of gastric carcinoma. It was negative in all conditions not associated with an ulcerative lesion of the stomach or adjacent duodenum. A positive test was found to have greater diagnostic significance than a negative one. In a more recent study the test was modified to

increase its objectivity (Rush¹⁰³). This procedure involved giving 200 cc. of 0.3% HCl, emptying the stomach 15 minutes later and, after an additional 15 minutes, introducing 200 cc. of a 2% solution of sodium bicarbonate. The accuracy of the test was increased by the fact that the patient was unaware of the expected characteristic response to each of the liquids in the presence of an ulcer. The procedure was found to be quite accurate in the differential diagnosis of ulcer from functional gastro-intestinal disturbances.

The physiologic factors which lower intragastric acidity in man are of obvious clinical importance. This acid reduction is brought about by the neutralizing effect of mucous and duodenal juices, and by dilution by admixed substances. A quantitative test has been devised to estimate the extent to which these factors are at work (Apperly and Cameron⁷). The method involves performing a fractional analysis using 250 cc. of 0.4% HCl as the test meal. The rate of reduction of acid concentration in the succeeding fractions was taken to be a measure of the rate of neutralization. It is the impression of several workers that this "acidity reduction test," as it is now known (Apperly and Cameron⁶), possesses greater diagnostic value than the routine fractional test meal, particularly since it produces more uniform results (Hollander and Penner¹¹¹).

Fluorescein. This dye has been applied in a test for determining the viability of the bowel (Herrlin, Glasser and Lange¹¹²; Boyd and Lange¹¹³). It is non-toxic in the required doses. The technique involves the rapid intravenous injection of 5 to 6 cc. of a 5% solution of fluorescein to which sodium bicarbonate has been added to make a 5% solution, while the questionable loop of bowel is observed under a dark purple light. The dye produces a characteristic golden green fluorescence of only those areas of bowel sufficiently supplied with blood. Areas which are out of circulation remain purple and the segments inadequately supplied are markedly diminished fluorescent.

possibility that the marked changes noted in the bowel could to a great extent be due to prolonged reflex vasospasm suggested the use of a combined procaine and fluorescein test. A 2% solution of procaine hydrochloride in doses up to 10 cc. was injected along the vessels leading to a strangulated loop. A return of normal color, peristalsis and fluorescence indicated restoration of the circulation. A follow-up of patients subjected to this procedure has confirmed its value.

Fluorescein is readily adsorbed onto ulcerated areas and develops a greenish fluorescent sheen. This observation was employed as an aid to gastroscopy. Ten cc. of a 1% solution introduced by tube produced a satisfactory fluorescence of ulceration over peptic ulcer and carcinoma (Robinson⁹⁹).

Barium Sulfate. The use of this opaque substance is standard in the roentgenologic examination of the alimentary tract. Various modifications of the usually employed mixture and its mode of administration have recently been explored with a view toward increasing its diagnostic scope. An opaque meal modified by the addition of gum acacia, cocoa and sugar, in specified proportions, permitted the simultaneous demonstration of the internal gastric topography together with most of the gastric contour (Rendrich and Poppel⁹⁶). It makes possible a study of the mucosa in a state of partial distention. In this respect it is superior to the double contrast method which often produces distention with ironing out of the rugal folds and diminution of mucosal relief. The modified opaque meal is especially useful in distinguishing between large benign and malignant ulcers on the lesser curvature. It is also of value in the early diagnosis of gastric pathology, in which the earliest changes are usually found on the mucosal surface.

In another study, after experimenting with various mixtures, it was found that a mixture containing 75 gm. of barium sulfate and 5 gm. of gum acacia in 2 quarts of water was most satisfactory in roent-

genography of the colon (Poppel and Bercow⁹⁰). Sufficient contrast was maintained and there was adequate semitransparency to enable visualization of any normal or abnormal luminal contents. This thinner mixture was particularly valuable for study of the colonic flexures and sigmoid. It was possible to resolve component loops in cases of colonic redundancy with relative ease by this method. Polypi and obstructions were well outlined and the thinner mixture occasionally filled diverticuli. When correlated with pathologic studies it was found that with this modified mixture the findings on Roentgen ray more nearly approximated the actual pathologic picture than did those with the routine thicker mixture.

By introducing a thinner, more dilute barium mixture through a duodenal tube directly into the small intestine, a better morphologic study was obtained than with any other available method (Schatzki¹⁰⁴). By observing the actual filling of the loops it was possible to judge better the extent and relationships of a lesion. Fistulæ and cicatrizing enteritis were frequently recognized as the barium spurted through the area of maximal narrowing. This method, using a fluid barium mixture, can be used to examine the ileocecal valve, cecum and ascending colon in instances where use of the barium enema is impossible or unsatisfactory.

The gastric emptying time was recently determined in 69 healthy males by the frequent fluoroscopic visualization of the stomach after administration of a test meal of cooked cereal mixed with barium sulfate. The mean gastric emptying time was 2.13 hours. The extremes ranged from 1.5 to 3.3 hours. Despite the unphysiologic nature of barium, it was felt that this method was preferable to frequent aspirations of gastric contents (Van Liere and Northup¹¹³).

Galactose. The speed of intestinal absorption in man can be tested by the galactose tolerance test (Althausen⁷). The test depends on the fact that this sugar, which is normally not found in the blood,

is rapidly absorbed into the blood stream when taken by mouth. The principle of the test involves determining the amount present in the blood at various intervals following its administration. A dose of 40 gm. of galactose in 400 cc. of water is given after an overnight fast and blood samples are tested quantitatively for galactose 30 and 60 minutes later. In normals, the highest peak varies from 13 to 30 mg. per 100 cc. Values below 10 and above 40 mg. were taken as abnormal. They were found to be greatly increased in hyperthyroidism and decreased in myxedema and Addison's disease. In another study the galactose absorption test was applied to various diseases in which impaired absorption is suspected (Beams, Free and Glenn¹¹). It was found to be subnormal in pellagra, non-tropical sprue, pernicious anemia and others. Galactose absorption also decreases with age (Meyer *et al.*⁷⁵). The significance of this procedure has been questioned as a test of absorption since changes in blood concentration may be due to changes in utilization, storage and excretion (Miller⁷⁶). The test is of questionable value in the presence of liver disorders. Since galactose is metabolized exclusively by the liver, high blood values occurring in the presence of liver dysfunction are more apt to be due to deficient utilization than to increased absorption (Bockus¹⁴).

PANCREATIC FUNCTION. *Secretin.* This protein substance was isolated in pure crystalline form in 1933 (Agren³) and was soon thereafter applied as a method of revealing the functional state of the pancreas (Agren and Lagerlöf; Agren, Lagerlöf and Berglund⁵). The basis of the method involves the maximal stimulation of the external secretory mechanism of the pancreas and the quantitative study of its response (Diamond and Siegel⁷). The total amount of pancreatic juice, the bicarbonate content and the content of amylase, trypsin and lipase are determined for the normal individual, and deviation from the normal serves as a means of detecting disorder of the pancreas. In

performing the test a dose of 0.75 mg. of pure secretin per kg. is injected intravenously. A double-lumen tube with one end in the duodenum and another about 10 inches higher in the stomach serves for the collection of specimens during a period of 1 hour after the injection. The curve of increased secretion rises to a peak in about 10 minutes and falls off gradually to the basal level in about 80 minutes. Simultaneous with the rise in volume of secretion there is a fall in the concentration of the enzymes; secretin has little or no influence on the secretory activity of the gland with respect to enzymes.

In the majority of instances the deviations from the normal apparently assume 1 or the other of 2 characteristic patterns (Lagerlöf,⁶⁹ Comfort²³). In the first type there is a hyposecretion of amylase without disturbances in secretion of the other components of the juice. This dissociated response is usually obtained in acute pancreatitis. However, it does not appear until at least 6 days after the onset of the illness. The secretin test cannot, therefore, be substituted for blood and urinary enzyme determinations in the early diagnosis of this disorder. The second type exhibits a non-dissociated impairment of function. There is a smaller response with respect to the volume of secretion as well as its enzyme and bicarbonate content. The bicarbonate fraction is least affected while the secretion of enzyme may vary considerably depending on the amount of tissue destruction and the degree of duct obstruction. This type of response is usually observed in carcinoma or fibrosis of the pancreas,⁷⁷ in chronic pancreatitis,⁵ and in pancreatic duct obstruction.²³ In this last disorder the volume response is negligible.²¹ If the volume response is negligible, the bicarbonate response is absent it can be assumed that only a small part of the external pancreatic function is complete.⁵ There is some indication that the amount of bicarbonate secreted during a test period serves as a more reliable indicator of pancreatic dysfunction than does the enzyme content.

The secretin test has yielded inconstant results in pancreatic carcinoma. In 1 report of 5 proved cases, the volume, alkalinity, and concentration of enzymes in the stimulated secretion were greatly reduced in all (Pratt⁹³), while in another study several cases yielded normal values.⁶⁹ The response to secretin is apparently determined by the location of the malignant process in the organ (Pollard, Miller and Brewer⁸⁹). Abnormal values were obtained when the lesion occurred in the head of the pancreas and normal values when located elsewhere.

In pancreatic fibrosis secretin fails to stimulate pancreatic flow (Maddock, Farber and Schwachman;⁷² Philipsborn *et al.*⁸⁷). A significant volume response, therefore, rules out pancreatic fibrosis regardless of how little enzyme activity is found on duodenal drainage.⁸⁷ The diminished volume and trypsin response in pancreatic fibrocystic disease differentiates it from chronic nutritional disturbances and celiac disease.^{72, 87} In steatorrhea the lipase response is deficient thus differentiating pancreatic steatorrhea from sprue (Diamond, Siegel and Myerson³⁰).

The secretin test serves as a means of testing pancreatic involvement in cases of gall bladder and liver disease.²⁹

Acetyl-beta-methylcholine Chloride. The use of this drug (methylcholine chloride of commerce) for testing pancreatic function is based on the fact that it is a stimulant to pancreatic enzyme secretion. In contrast to secretin which tests the humoral mechanism, this substance explores the vagal mechanism of pancreatic secretion²³ (Comfort and Osterberg²⁴). A subcutaneous dose of 15 mg. of acetyl-beta-methylcholine chloride provokes a secretory response characterized by a small increase in volume, no significant change in hydrogen ion concentration, and a prolonged increase in the concentration of enzymes. The procedure is carried out in a manner similar to the secretin test. In acute pancreatitis the characteristic dissociated response which followed secretin stimulation is also seen after stimulation with

acetyl-beta-methylcholine chloride. With pancreatic tissue destruction and pancreatic duct obstruction there occurs a drop in both volume and enzymes to below the normal range. The chief value of this drug in pancreatic study is that it permits a more complete study of enzyme secretion than is possible with secretin.

Acetyl-beta-methylcholine chloride combined with secretin or eserine sulfate may produce changes in the serum enzymes that are dependent on the state of the pancreas. This fact has been applied as a means of evaluating pancreatic function (Popper and Necheles;⁹¹ Popper, Olson and Necheles⁹²). A combination of acetyl-beta-methylcholine chloride and eserine sulfate injected subcutaneously into the experimental animal produced a rise in serum amylase and lipase in the presence of a normal pancreas, but not if the gland was atrophic.⁹¹ When acetyl-beta-methylcholine chloride and secretin were used as the stimulant there was no change in serum enzyme levels in the normal animal, but a rise in serum lipase occurred with obstruction of the pancreatic duct.⁹² The suggestion is made that the latter test might be successfully applied to human subjects for the diagnosis of such disorders as functional insufficiency, chronic pancreatitis or pancreatic fibrosis.

GALL BLADDER FUNCTION. Secretin. The gall bladder normally stores the bile secreted by the liver with the result that duodenal juice may be collected substantially free of liver bile. If the gall bladder fails to function, bile contaminates the duodenal (pancreatic) juice. This has been made the basis of a test for gall bladder function (Diamond, Siegel and Myerson³¹). A tube is inserted into the duodenum and samples of juice are collected at 10 and 20 minute periods for 80 minutes after an intravenous dose of secretin which causes a discharge of bile from the liver (60 to 80 cc.). The samples are tested for bile by the icteric index method. In the normal, the samples become free of bile 5 to 10 minutes after the injection, in common duct obstruction

they remain free of bile, in a non-functioning gall bladder they remain strongly contaminated with bile. This test may help to establish the presence of a normal gall bladder when the failure to concentrate dye may suggest a pathologic organ.

Magnesium Sulfate. In the diagnosis of gall bladder disease it is often necessary to stimulate the gall bladder to contract and study the ability of the gall bladder to empty. Magnesium sulfate in a dose of 30 cc. of a saturated solution causes the gall bladder to contract and the sphincter of Oddi to relax. The effect is similar to that of egg yolk but is less marked. The mechanism of this action (Boyden, Bergh and Layne¹⁶) is probably hormonal, the magnesium sulfate being absorbed, causing the formation of a cholecystokinin-like substance.

Sodium Phenoltetraiodophthalein. This substance (iso-iodeikon of commerce) is a cholecystographic agent. It is preferred to sodium tetraiodophenolphthalein for intravenous use (Graham, 1930) because it stains the blood serum and its retention can be used as a liver function test while its appearance in the gall bladder serves as a contrast medium. A dose of 2.5 gm. in 5% solution is slowly injected intravenously by gravity and a sample of blood is taken 30 minutes after the middle of the injection period. A retention of 50% or more has been considered a poor operative risk (Graham). The value of this combined test is doubted from other experiences (McIntyre¹⁷). Several cases of severe reaction also occurred due to systemic action and locally from tissue infiltration.

Sodium Tetraiodophenolphthalein. This dye may be used for either oral or intravenous cholecystography. Attention has been paid to increased dosage and double dose oral techniques as means of increasing the density of the shadow after tetraiodophenolphthalein. Dosage seems to be the deciding factor (Feldman¹⁸), since if it is large enough, the shadow is the same whether the compound is taken at one time or in 2 fractions, from 6 to 12 gm.

depending on the patient's weight. The incidence of nausea, vomiting and diarrhea is not significantly different for the 2 methods.

Several factors influence the degree of visualization of the gall bladder with contrast dyes, namely the mode of administration of the dye, its absorption, the dosage, the function of the liver in excreting the dye, the ability of the gall bladder to empty and hence to fill (Sosman¹⁹), the function of the gall bladder in concentrating the dye, and a normal sphincter of Oddi mechanism (Feldman²⁰). From a study of a large group of cases with sodium tetraiodophenolphthalein, it was found that the intravenous method (about 3 gm. in 400 to 600 cc.) and the double dose oral method (4 gm. in dose repeated 24 hours later) gives a higher incidence of visualization than the single oral dose (DeLor *et al.*²¹). A gall bladder which is not visualized by the former 2 tests is pathologic.

In the small percentage of cases in which the routine 18 to 24 hour study with single, double and triple dose methods fails to reveal, or only faintly outlines the gall bladder, an intensified procedure lasting 42 to 48 hours may succeed in visualizing the gall bladder. A prolonged period of dye concentration is apparently necessary in some instances (Feldman²¹). By performing cholecystography just prior to surgical removal of the gall bladder it was possible to correlate the density of the shadow with the iodine content of its bile (Joffe and Wachowski²²). A concentration of at least 0.25% (2.5 mg. of iodine per gm. of bile) was necessary for faint visualization, with 0.39 and 0.89% necessary for moderate and dense shadows respectively.

Priodax. Priodax is a proprietary name for beta-(4-hydroxy-3,5-diiodophenyl)-alpha-phenyl propionic acid, a preparation used in oral cholecystography (Kober²³). The usual procedure is a total dose of 3 gm. taken after a light supper and a Roentgen ray 15 hours later. The 3 gm. dose, however, is not

always suitable, particularly in cases with borderline shadows. A 4 gm. dose was found more likely to give a satisfactory shadow, and has been advised for routine use (Collins and Golden²²). In another study the drug was given by single (3 gm.), divided (3 gm. total) and double dose (3 gm. each after lunch and supper) techniques. A comparison of these methods indicated the single dose method as the one of choice (Vaughan and Eichwald¹²⁰).

Priodax is excreted in the urine (Junkmann⁶⁶). This accounts for the burning on urination seen in approximately 4% of patients taking this drug.²² It is inadvisable to use priodax in individuals with impaired renal function, particularly when accompanied by nitrogen retention (Ochsner⁶³). In a preliminary study of kidney function, there was no evidence that the drug causes kidney damage.²²

It is uniformly agreed that the greatest advantage in the use of this drug is the absence of opaque material in the colon, particularly the hepatic flexure.^{17,22,27,122} The impression of many observers is that priodax produces shadows that are at least equally as good if not better than those obtained with tetraiodophenolphthalein.^{27,35} A surgical follow-up of Roentgen ray findings with priodax in a large series indicated its diagnostic accuracy to be over 96%. This compares favorably with tetraiodophenolphthalein (Hefke⁶⁶).

The most frequent toxic symptoms seen with priodax are nausea, vomiting, diarrhea, abdominal cramps, headache, burning in the throat (local effect) and burning on urination. In general they are very mild and pose no hazard to the patient (Dannenberg²⁷). The drug is considered less toxic than tetraiodophenolphthalein (Marshall⁷³).

The gall bladder emptying time in response to a fat meal is slower with the priodax-filled vesicle than with tetraiodophenolphthalein. This is of some importance for in failing to contract down to the small sized partially filled vesicle obtained with tetraiodophenolphthalein, a distinction between gas shadows and small

opaque stones cannot as readily be made. In addition, the rapid and vigorous response to a fat meal by the tetraiodophenolphthalein filled vesicle may permit visualization of the hepatic ducts and its radicles. This may aid in the diagnosis of biliary dyskinesia. Priodax on the other hand appears to hinder gall bladder emptying and visualization of the ducts (Copleman²⁵).

EPILEPSY. *Pitressin.* The fact that water retention tends to precipitate epileptic seizures in a susceptible individual and the fact that pituitary extract promotes water retention by its antidiuretic action have been applied in a diagnostic test for epilepsy (Blyth¹³). It is particularly applicable to such cases in which the diagnosis is difficult because of infrequent spontaneous seizures, or because of the need for differentiation. The test takes 72 hours or less. The subject receives abundant water until his weight increases by about 2%; usually in about 48 hours. Pitressin is then injected intramuscularly in an initial dose of 0.25 cc. and in 0.5 cc. doses every 2 hours, and 300 cc. of water orally until a convulsion is produced. It is rarely necessary to give more than 10 doses. In a series of 87 adult cases, the subsequent course corresponded to the result of the test in 86.6% of the tests. Others also report the test to be of diagnostic value.^{43,52,64} The procedure is not without danger and deaths during the test have occurred. Autopsy revealed marked cerebral damage.^{63,79} It is suggested that these might be avoided by reversing the process of water retention as soon as a convulsion occurs by rapid dehydration with intravenous hypertonic glucose solution and mercurial diuretics.

Metrazol. The fact that epileptics have a lower convulsive threshold to central nervous system stimulation than do non-epileptics has been made the basis of a diagnostic test for epilepsy (Jan⁶⁴). The test involves the intravenous injection of 3 to 6 cc. of a 10% solution (300 to 600 mg.) of metrazol, and the occurrence of an epi-

leptic seizure soon thereafter has been taken as proof of susceptibility to epilepsy. The majority of seizures occurred within 1 minute following the injection. In this study the occurrence of convulsions in non-epileptics was sufficiently frequent to render the test of doubtful value. In another report, seizures occurred in 87% of the epileptics and in 36% of the non-epileptics.¹⁰ Others, however, have reported the test as reliable (Goldstein and Weinberg⁴⁹).

MYASTHENIA GRAVIS. Neostigmine. The fact that parenteral neostigmine produces striking relief of the characteristic muscular weakness of myasthenia gravis, has led to its application in a diagnostic test for this disorder (Schwab and Viets;¹⁰⁶ Viets¹²¹). The procedure involves the intramuscular injection of 1.5 mg. of neostigmine methylsulfate, to which 0.6 mg. of atropine sulfate has been added, and the observation of the test response at 10 minute intervals for 1 hour. Rapid improvement in the subjective, and particularly the objective symptoms of this disorder, *e. g.*, ophthalmoplegia, dysarthria, dysphagia, facial paralysis and general voluntary muscle weakness, is diagnostic. Little or no response to the test dose rules out myasthenia gravis. A system of scoring the subjective and objective improvement has been devised.¹⁰⁶ The test is of particular value in the obscure case of myasthenia gravis, and in the differential diagnosis from disorders simulating it, *e. g.*, progressive muscular dystrophy, psychoneurosis and fatigue states, multiple sclerosis, cranial nerve palsies, etc. The test is considered to be almost 100% reliable.^{17,123} Numerous studies have confirmed its accuracy (Harvey and Whitehill,⁵¹ Thorner and Yaskin,¹¹³ Gammion and Scheie⁶⁷). A modification of the test has been explored with a view to increasing its objectivity. The response of the pharyngeal muscles to neostigmine was studied by roentgenoscopic examination of the pharynx after ingestion of a liquid barium mixture. Involvement of the muscles was indicated by retention of

the mixture in the pyriform sinuses in excessive amounts. On reëxamination of the pharynx 15 minutes after a test dose of neostigmine, the ability to swallow had returned to normal in myasthenia gravis. It failed to return when due to other causes, *e. g.*, progressive bulbar palsy, etc. (Schwab and Viets¹⁰⁷).

Quinine. The fact that quinine aggravates the muscular weakness of myasthenia gravis, in contrast to the beneficial effect of neostigmine, has been applied in a combined test to those obscure cases of myasthenia gravis in which the neostigmine test produces an equivocal response (Harvey and Whitehill;⁵⁵ Eaton⁵¹). Quinine sulfate given in 2 doses, each 0.65 gm., 2 hours apart, produces a weakening effect within 2 hours after the second dose in an individual with this disorder. After aggravating the symptoms, the beneficial response to neostigmine is more clearly defined.⁵⁵

Curare. Patients with myasthenia gravis are more sensitive to curare than are normal individuals. Approximately one-twentieth of the dose necessary for mild generalized curarization in normal individuals is effective in producing this response in myasthenics (Bennett and Cash¹²). In the questionable case, curare in a dose of 0.05 mg. per kg. of body weight injected intravenously over a 1 minute period will produce a peak response within 2 minutes following the injection. A positive test response is indicated by aggravation of the already present myasthenic symptoms. The reaction is terminated within 2 to 3 minutes by neostigmine methylsulfate (1.5 mg.) and all acute effects of curare disappear within a minute. The dose of curare employed is ineffective in producing curarization in other motor disorders, *e. g.*, post-encephalitic parkinsonism, psychoneurosis, etc., at least; therefore, rules out such conditions.¹¹

Inasmuch as neither the quinine nor curare tests are entirely without danger, since either may precipitate a myasthenic crisis, it is inadvisable to resort to them

until after the neostigmine test has failed to give the necessary information.

PREGNANCY. *Neostigmine.* Premenstrual uterine hyperemia appears to be associated with acetylcholine liberated in response to estrogen stimulation. It was suggested, therefore, that the potentiation of naturally occurring acetylcholine, by means of the cholinesterase inhibiting substance, neostigmine, might be effective in inducing menstruation in those cases of menstrual delay due to lack of vascular response, and ineffective when due to endocrine dysfunction or pregnancy. The failure of neostigmine to initiate menstrual flow in the experimental animal when delay was due to early pregnancy, indicated its probable safety in human application. On this basis a pregnancy test was devised (Soskin, Wachtel and Hechter¹¹¹). The procedure involves the intramuscular injection of neostigmine methylsulfate (1:2000) in doses of from 1 to 3 cc. on from 2 to 4 successive mornings. The failure to menstruate soon thereafter is taken to indicate pregnancy. The test is applicable only where the menstrual history has been normal up to the last period, *e. g.*, cases of "nervous" amenorrhea or pregnancy. The test is not suitable where there have been menstrual disorders due to endocrine imbalance, local pathology, or organic disease elsewhere.

In a study of 23 women in early pregnancy, and in 25 with delayed menstruation in which pregnancy was ruled out by other methods, including the Friedman test, the neostigmine test yielded a correct result in every case. It compared favorably with the Friedman test.¹¹¹ Numerous studies followed the original report, with most workers using a 2 cc. dose of the drug on from 1 to 3 successive mornings. In most instances the test was reported as being close to 100% accurate and as reliable as the Aschheim-Zondek and Friedman tests.^{112,113,114,115} In 1 report, however, discrepancies in both positive and negative results were obtained, and attention was called to the danger of inducing abortion with neostigmine (Winkelstein¹¹⁶).

Another disadvantage exists in the fact that it is not always possible to rule out the many factors such as local pathology, endocrine disturbances, and other disorders which may be the cause of a missed menstrual period (Weisman¹²³). Inasmuch as bleeding in several instances of pregnancy have occurred with this test,¹²⁶ the risk of abortion that goes with its use seems unnecessary, particularly since such accurate procedures as the Aschheim-Zondek and Friedman tests are readily available.

Chorionic Gonadotropin. The absence of the chorionic gonadotropic principle of pregnancy urine in the non-pregnant woman suggested that she might react to its intradermal injection, while the pregnant woman, already possessing it, would not. The procedure involves the intradermal injection of 2 minims (0.12 cc.) of this substance (antuitrin-S of commerce) into the forearm. A reading is taken $\frac{1}{2}$ hour later and if a slight reaction is present it is read again at the end of the hour. The positive reaction is taken as an area of erythema around the injection site measuring 7 to 40 mm. It has been concluded that the test is more sensitive than the Aschheim-Zondek test (Gilfillen and Gregg⁴⁴), although the controls seem to be inadequate. The evidence of carefully controlled studies (Schneider and Cohen,¹⁶⁵ Hadley⁵³) leaves no doubt that this test is unreliable.

Placental Extract. The suggestion that the placenta may produce a protein-like foreign substance which, when used as an antigen in the non-pregnant woman, will produce an allergic reaction, was applied as a test for pregnancy (Gruskin⁵²). One-tenth cc. of the antigen is injected intradermally and in positive cases a slight area of inflammation with pseudopodia appears. In pregnancy no such reaction appears.⁴² The test was thought to be of great value where a quick diagnosis was necessary, as in the differential diagnosis of ectopic pregnancy from other conditions simulating it. In another study (Schwartz¹¹⁷), the test was found to be

correctly positive in 96% of the pregnant and 90% of the non-pregnant women. Disturbed endocrine function and allergic hypersensitivity are sources of error. Also, experience in interpreting skin tests is necessary.¹⁰³

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RADIOLOGY

UNDER THE CHARGE OF

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ROENTGENOLOGIC DIAGNOSIS OF CERTAIN CONGENITAL LESIONS OF THE HEART AND GREAT VESSELS

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UNTIL recently it was often considered unnecessary to determine the exact nature of the cardiac abnormality in cases of congenital heart disease since nothing could be done to alter the anomaly. Great advances in thoracic and vascular surgery have changed this situation.

In 1939, Gross and Hubbard¹⁰ described the successful surgical ligation of a patent ductus arteriosus. In the following year, Touroff and Vesell²⁰ reported the surgical ligation of a patent ductus arteriosus which was the site of subacute bacterial endarteritis. The patient was relieved not only of the cardiac disability but also of the subacute bacterial endarteritis. As a result of the success achieved by Gross and Hubbard, and Touroff and Vesell, the surgical ligation of patent ductus arteriosus was attempted by other surgeons and proved to be a most satisfactory surgical procedure. Ligation of a patent ductus arteriosus is recommended not only in cases in which patients are disabled by the cardiac abnormality but also as a measure to prevent or eliminate subacute bacterial endarteritis which often is a complication.

In 1945, Blalock and Taussig³ reported a successful operation for congenital malformations of the heart associated with pulmonary stenosis or atresia. The abnormality is encountered most frequently as one of the components of the tetralogy of Fallot, which will be described later. In brief, the surgical procedure consists of producing an artificial ductus arteriosus by anastomosing one of the subclavian

arteries, the common carotid artery, or the innominate artery with one of the pulmonary arteries. In this way, an adequate pulmonary circulation is obtained. More recently, Blalock² reported the results of this operation in 57 cases, and this report undoubtedly will lead to a more widespread use of this surgical method.

Crafoord and Nylin,⁴ in October 1945, described a successful surgical operation for coarctation of the aorta. In 2 cases they were able to excise the stenosed segment of the aorta and anastomose the divided ends of that vessel, thus allowing a normal systemic arterial flow. Later in the same year, Gross,³ by using a similar technique, operated on 2 patients who had coarctation of the aorta. Since then, Crafoord and Nylin, and Gross have operated on other patients who had this condition but they have not published their results. It seems that this is a most logical operation despite the difficulty of the procedure.

Great advances have thus been reported for the relief of certain congenital abnormalities of the heart and great vessels. The benefits obtainable from these truly great discoveries are dependent primarily on the accurate diagnosis of congenital heart disease. The diagnosis of some congenital heart diseases can be made accurately by properly trained physicians. This is shown by the fact that in those cases in which operation was performed for patent ductus arteriosus, pulmonary stenosis (especially tetralogy of Fallot) and coarctation of the aorta, the pre-

operative diagnosis was surprisingly accurate. The clinical diagnosis of these conditions is based on history, symptoms, localization and transmission of murmurs and thrills, the presence or absence of cyanosis, circulatory studies, electrocardiographic changes and roentgenologic observations.

The purpose of this paper is to review the roentgenologic diagnosis of those cardiovascular abnormalities that are amenable to surgical treatment. Anatomic, physiologic and clinical aspects are far too complex to be considered in detail and will be touched on only as briefly as is necessary to explain the roentgenologic findings. With regard to congenital heart disease in general, it may be stated that roentgenologic observations have the following results: (1) They may be of no assistance in diagnosis; (2) they may furnish confirmatory evidence of a condition already suspected clinically; (3) they may cause doubt as to the tentative clinical diagnosis and suggest some other or additional congenital abnormality; (4) they may provide a definite diagnosis as to the type of cardiovascular abnormality; (5) they may provide useful information as to anatomic relations if operation is contemplated.

It is erroneous to think that there is such great variety and combination of congenital lesions of the heart that an attempt to make an accurate diagnosis is useless.⁶ This impression has been obtained from postmortem material that has included a rather large number of newborn infants who have died of congenital heart disease. Infants who die of congenital cardiac disease obviously have lesions that are incompatible with life and these hearts are, therefore, greatly distorted. If lesions that are completely incompatible with life are excluded, one finds that most cardiac abnormalities are not extremely complex. In many instances in which the patients have survived infancy, a rather accurate estimate can be made as to the type of cardiac abnormality that is present.

Sussman's¹⁸ classification of cardiovascular abnormalities should be useful to radiologists. This classification is as follows:

1. OBSTRUCTION OF THE OUTFLOW TRACTS.

(a) Isolated pulmonic stenosis. (b) Aortic or subaortic stenosis. (c) Coarctation of the aorta.

2. INTRACARDIAC SHUNTS. (a) Isolated interventricular septal defect. (b) Interatrial septal defect. (c) Tetralogy of Fallot. (d) Eisenmenger's complex.

3. EXTRACARDIAC SHUNTS. (a) Patent ductus arteriosus. (b) Arteriovenous aneurysm.

4. MISCELLANEOUS ANOMALIES. (a) Positional changes in the heart and great vessels. (b) Idiopathic dilatation of the pulmonary artery. (c) Congenital aortic aneurysm. (d) Aberrant pulmonary veins, usually in association with interatrial septal defect. (e) Anomalous origin of 1 coronary artery, which is usually associated with left ventricular dilatation and hypertrophy. (f) So-called congenital hypertrophy from which von Gierke's disease must be distinguished.

This classification is not intended to include all the varieties of cardiac abnormalities that might be encountered by pathologists. It does include most varieties of congenital heart disease that are compatible with life and it is only with these that radiologists are concerned.

With regard to the diagnosis of patent ductus arteriosus, it may be stated that in most instances the diagnosis can be made accurately without using roentgenologic methods. The so-called machinery murmur and the associated thrill are almost pathognomonic of this condition. Roentgenologic examination is used, as a rule, to obtain evidence to confirm the clinical diagnosis and to help rule out the presence of other cardiac abnormalities; roentgenography and roentgenoscopy cannot alone lead to an absolute diagnosis of patent ductus arteriosus.

Patent ductus arteriosus may be found associated with other cardiac abnormalities, but many of these combinations are not compatible with life. It is considered that, in older children and in adults, patent ductus arteriosus is found most frequently

as an isolated defect or is associated with a minor cardiac abnormality. The success of surgical ligation in many cases of patent ductus arteriosus has substantiated this concept.

The experimental work of Eppinger, Burwell and Gross⁶ has shown the effects of a patent ductus arteriosus on the heart and circulation, and, to a limited extent, roentgenologic observation reveals these effects. They found that 45 to 75% of the blood leaving the left ventricle enters the pulmonary circulation by way of the shunt between the aorta and the pulmonary artery. As the result of this, the left ventricle must pump 2 to 4 times as much blood as the right ventricle does. Certainly this must depend somewhat on the size of the patent ductus arteriosus but there can be no doubt that in all instances there must be an increased volume of blood in the pulmonary circulation and an unusual load on the left ventricle.

Eppinger and his associates said that the heart is affected in various ways and these have been summarized by Donovan, Neuhauser and Sosman⁵ as follows: (1) There is left ventricular enlargement as the result of a greatly increased output. (2) With each beat an increased volume of blood passes through the left ventricle and pulmonary artery, causing increased pulsation of these structures. (3) There is prominence of the pulmonary artery since it receives blood from both the right ventricle and the aorta. (4) Increased flow in the branches of the pulmonary artery causes excessive pulsation of these structures; this is called "bilar dance." (5) The extra volume of blood in the lesser circulation leads to vascular congestion and finally "if a normal mitral valve is not large enough to transmit this large volume of blood without an elevated left auricular pressure, there may be visible dilatation of the left auricle."

Donovan, Neuhauser and Sosman have reviewed the roentgenologic findings in the thorax in 50 cases of isolated patent ductus arteriosus in which operation was performed subsequently. They gave the

following criteria for the diagnosis of this condition:

1. *There is enlargement of the left ventricle.* The heart usually appears normal in size or slightly enlarged. When enlargement is seen it is due to hypertrophy of the left ventricle. If patent ductus arteriosus is complicated by subacute bacterial endarteritis, the heart may be greatly enlarged.

2. *Evidence of right ventricular hypertrophy is more suggestive of more severe congenital heart disease.* It did not occur in the cases of patent ductus arteriosus reported by these authors.

3. *The left auricle is enlarged.* They found this to be one of the most common signs of patent ductus arteriosus. This enlargement was demonstrated roentgenologically by showing the indentation of the barium-filled esophagus by the left auricle in the right anterior oblique view. Mitral stenosis will cause enlargement of the left auricle but they stated that this is seldom confused clinically with patent ductus arteriosus. Auricular fibrillation is found in cases of mitral stenosis but virtually never occurs with patent ductus arteriosus. Calcification of the mitral or aortic valve would indicate rheumatic heart disease.

4. *The pulmonary artery is dilated.* This is one of the most frequent findings in cases of congenital heart disease and is present in most cases of patent ductus arteriosus. They found that this could best be demonstrated in the right anterior oblique view, which shows best the relief of the true pulmonary conus. Great enlargement was not seen unless there was an associated bacterial endarteritis. They said that a very large pulmonary artery, especially if associated with a definitely enlarged heart, is more suggestive of Eisenmenger's complex, interauricular septal defect or Lutembacher's syndrome.

5. *Exaggerated beat of the left ventricle and of the pulmonary artery can be seen by roentgenoscopy.* This was seen in two-thirds of the cases reported by these authors. The characteristic pulsation in

cases of patent ductus arteriosus is the exaggerated systolic pulsation of the aorta and pulmonary artery, which occurs simultaneously with the exaggerated ventricular contraction. This cannot be demonstrated so well by kymography as by roentgenoscopy. These authors stressed that this exaggerated beat can be distinguished from that due to other conditions.

6. *There may be hilar dance*, which is a systolic expansion of the large vessels in the hila of the lungs. These authors found this in a third of their cases. This is seen in other congenital lesions of the heart, especially with interauricular septal defect.

7. *Engorgement of the pulmonary vessels may be seen*. This is usually not severe and is seen most frequently when subacute bacterial endarteritis is present.

These findings by Donovan and his associates are similar to those described by other authors. The only point about which there is much controversy has to do with enlargement of the left auricle. This will be considered later. Roesler¹² and Sussman said that there may be some demonstrable enlargement of the right ventricle but they agreed that this enlargement should not be very great. Eppinger, Durrell and Gross reported no evidence of right ventricular hypertrophy. Most authors emphasize that there should not be much cardiac enlargement unless subacute bacterial endarteritis is present. It has been mentioned by Roesler and Sussman that at times a patent ductus arteriosus will overshadow the "aortic window" in the left anterior oblique view but this has not been seen very frequently. Roesler stated that there occasionally is an isolated plaque of calcium in the aorta at the point of attachment of the ductus arteriosus and that this may be seen roentgenologically. Aneurysmal dilatation of the ductus arteriosus or of the pulmonary artery may occur and this might tend to confuse the roentgenologist in some cases. Sussman, Grishman and Steinberg¹³ have emphasized that in cases of patent ductus arteriosus the pulsation

of the pulmonary vessels at the hila is different from that which is seen in cases of interauricular septal defect. In the latter condition, the vessels are much larger and the pulsation is greater, since there is an associated pulmonary insufficiency, organic or relative, which causes a collapsing type of pulsation, which these authors preferred to consider as a true hilar dance.

Angiocardiography is of definite value in the diagnosis of patent ductus arteriosus. Steinberg, Grishman and Sussman¹⁷ have summarized the angiocardiographic findings as follows: (1) There is a distinctive localized dilatation of the descending aorta just beyond the isthmus. The diameter of the aortic end of the ductus arteriosus is usually 2 or 3 times that of the pulmonic end. The localized dilatation seen by angiocardiography may be the aortic end of the ductus arteriosus or it may be a traction aneurysm of the aorta caused by the attachment of the ductus arteriosus. A small bulge or a more or less uniformly dilated segment may be seen. It projects toward the main and left pulmonary arteries. It is best seen in the left anterior oblique view but is also seen in the left lateral and right anterior oblique views. (2) The main and left pulmonary arteries are elevated; the appearance suggests that they are drawn toward the isthmus of the aorta. (3) The main and major branches of the pulmonary artery are dilated. (4) Varying degrees of left ventricular dilatation are present.

The localized dilatation of the aorta seems to be a reliable diagnostic sign and was not seen by Steinberg and his associates in any other condition than patent ductus arteriosus. They did not find with angiocardiography that persistent opacity in the pulmonary vascular tree was a reliable evidence of patent ductus arteriosus. The ductus arteriosus itself, especially its length and caliber, could not be demonstrated by angiocardiography. Steinberg and his associates found no evidence of left auricular enlargement, and thus their observations conflict with those of Donovan,

Neuhauser and Sosman, and Eppinger, Burwell and Gross. Most authors^{7,12,13,17,18} do not mention enlargement of the left auricle as a common finding in cases of patent ductus arteriosus. It is important that further observations clarify this disputed point.

According to Steinberg and his associates,¹⁷ the following conditions may be mistakenly diagnosed as patent ductus arteriosus: (1) interauricular septal defect; (2) idiopathic dilatation of the pulmonary artery; (3) Eisenmenger's complex; and (4) pulmonic insufficiency due to absence of a cusp. In all of these conditions, there is enlargement of the pulmonary artery.

In cases of interauricular septal defect, the heart is almost always greatly enlarged; this enlargement is greatest to the left but there is often moderate enlargement to the right. The right auricle and ventricle are greatly enlarged. There is tremendous enlargement of the pulmonary arteries and the peripheral pulmonary vessels. The left auricle is not much enlarged unless there is associated mitral valvular disease (Lutembacher's syndrome). There is a definite "hilar dance." The aortic knob is very small or cannot be seen. By angiocardiology, the large right auricle can be easily seen and the greatly dilated pulmonary arteries also can be demonstrated. The current in the intracardiac shunt is from left to right and cannot be shown by angiocardiology unless there is a reversal of the flow, as may occur with cardiac decompensation. Owing to the rapidity of the injection when angiocardiology is used, there is increased pressure in the right auricle. This rarely may cause a temporary flow from right to left, which may be seen.

In cases of idiopathic dilatation of the pulmonary artery, there is no enlargement of any of the cardiac chambers. Pulmonary insufficiency due to absence of a cusp can produce a "machinery" murmur. Angiocardiology might be necessary to make the correct diagnosis. The diagnosis

of Eisenmenger's complex will be considered with the tetralogy of Fallot.

The value of roentgenologic observations in cases of patent ductus arteriosus varies greatly. In most instances it serves only to demonstrate that the cardiac silhouette and size and the appearance of the great vessels are consistent with a well-established clinical diagnosis. Shapiro¹⁴ said that roentgenologic studies are of value not only in aiding in the diagnosis but also in observing the progress in individual cases. He said that roentgenologic examination will reveal the severity of the leak and the amount of cardiac strain that is present. According to Shapiro, if the patent ductus arteriosus is small, the heart will be more nearly normal in size, whereas if the shunt is large, the heart will be larger and the pulmonary arteries greater in size. In some cases, roentgenologic observations will raise a doubt as to the validity of the diagnosis of patent ductus arteriosus or will suggest the presence of some additional congenital cardiac anomaly. In such instances, angiocardiology can be of greatest assistance. It has been stated that patent ductus arteriosus can be present when no machinery murmur is heard.^{7,11} In such cases, the diagnosis can only be made with certainty by means of angiocardiology. It is unfortunate that angiocardiology does not show the length and caliber of the ductus arteriosus since this information would be of great value to the surgeon if ligation were contemplated.

Stenosis or atresia of the pulmonary artery is at times an isolated cardiac defect, but most frequently it is encountered in combination with interventricular septal defect, dextroposition of the aorta and hypertrophy of the right ventricle. These anomalies constitute the tetralogy of Fallot. Stenosis of the pulmonary artery prevents an adequate pulmonary circulation and may cause death or gravely impair health. Recognizing this as a primary evil in many cases of congenital abnormalities of the heart, Blalock and Taussig conceived and successfully

developed the artificial ductus arteriosus operation. By diverting blood from a main branch of the aorta to the pulmonary artery, an adequate pulmonary circulation was obtained. Up to the present time, almost all of the patients who have undergone this operation have had the tetralogy of Fallot. Blalock stated, however, that the operation is indicated in cases of pulmonary atresia with or without dextroposition of the aorta and with or without defective development of the right ventricle, in cases of truncus arteriosus with bronchial arteries, and in cases of single ventricle with a rudimentary outlet chamber in which the pulmonary artery is diminutive in size. The operation is not indicated in cases of complete transposition of the great vessels, or in cases of Eisenmenger's complex, and probably not in cases of aortic atresia.

On roentgenograms of the thorax, the findings in cases of the tetralogy of Fallot are: (1) absence of fullness of the normal pulmonary conus; (2) the shadow at the base of the heart to the left of the sternum is concave and not convex—concave shadow in this region, in cases of persistent cyanosis, always means that the pulmonary artery is misplaced, absent or diminutive in size; (3) the pulmonary window appears abnormally clear in the left anterior oblique view; (4) the heart frequently may appear normal in size, but sometimes it will seem to be moderately enlarged, and in such instances this is due to hypertrophy of the right ventricle—as the result of right ventricular hypertrophy, the heart is enlarged to the left and the apex is lifted from the diaphragm and has a blunt appearance. This cardiac silhouette has been called “*cœur-en-sabot*”; (5) it has been stated that dextroposition of the aorta can be seen in conventional roentgenograms. This may be true but it is not likely to be a very reliable observation, and in many instances dextroposition of the aorta is not suggested by the roentgenogram.

Angiocardiography can be of great assistance in the diagnosis of pulmonary

stenosis and its associated cardiac abnormalities.^{16,18,19} In cases of tetralogy of Fallot, angiocardiography reveals simultaneous opacity of the aorta and of the pulmonary artery. Sometimes the density of the medium in the aorta is great enough to show the entire aorta and its major branches. The caliber of the pulmonary artery can be determined. The diameter may be so small that the artery cannot be identified in its entire length. The stenosed portion of the pulmonary artery or conus arteriosus can usually be identified. The right auricle and ventricle can be seen to be enlarged. Aortic anomalies can be seen if present.

Blalock and Taussig warned against operating in cases in which the pulmonary artery is prominent or when there are pulsations at the hila. They also stated that there must be clinical and roentgenographic evidence of absence of pulmonary congestion. They further said that the only situation in which there is absence of the normal shadow of the pulmonary artery with adequate pulmonary circulation is complete transposition of the great vessels with the pulmonary artery lying behind the aorta. As the result of this, in the antero-posterior view, the aortic shadow is narrow and there is a concave curve at the base of the heart to the left of the sternum. In the left anterior oblique view, the 2 vessels lie side by side and therefore the shadow cast by these great vessels is increased in width and the pulmonary window is not abnormally clear. Although there is no pulsation at the hila, pulmonary congestion frequently develops.

A right aortic arch occasionally occurs with the tetralogy of Fallot. The importance of this has been stressed by Blalock and Taussig since the major branches of the aortic arch are altered in position when this occurs and the surgical approach must be changed. The variations of right aortic arch have been described by Bedford and Parkinson¹ and by Sussman. In all types, the aorta arches over the root of the right lung rather than over the root of the left

lung. In most instances there is then a "high-crossing" and the aorta abruptly turns to the left as soon as it has crossed the right main bronchus. In going to the left of the spinal column, it goes behind the esophagus, displacing it and the trachea forward. Behind the esophagus there is a dilatation or diverticulum of the aorta which is caused by a persistence of the distal portion of the left fourth embryonic arterial arch, and the left subclavian artery arises from this. The aorta may then descend in the thorax on either the left or right side of the spinal column. If it descends on the right side, there will be a secondary "low-crossing" so that it can reach the aortic hiatus. This, the usual type of right aortic arch, is easily recognized roentgenologically since the aortic knob can be seen to be on the right, the barium-filled esophagus is indented on the right by the aortic knob in the antero-posterior view, and the aortic arch displaces the barium-filled esophagus forward in the right anterior oblique view. The course of the descending aorta may also be seen and noted if it is on the right side.

There is a less common type of "high-crossing" right aortic arch which is more difficult to identify. In this case, an artery arises from the ascending aorta and crosses in front of the trachea to the left side. This is a persistence of the proximal part of the fourth embryonic arterial arch and it serves as a left innominate artery. The right subclavian and right common carotid arteries arise separately from the aortic arch. In such cases, the aorta crosses to the left of the spinal column as soon as it crosses the right main bronchus but it does not go behind the esophagus and there is no aortic diverticulum. The roentgenologic diagnosis is more difficult as the result of this; there is indentation of the barium-filled esophagus on the right side in the antero-posterior view and there is a slight backward displacement of the esophagus in the left anterior oblique view. Angiocardiography will show this type of right-sided aortic arch when it is combined with the tetralogy of Fallot

and in some instances the correct diagnosis might be very difficult without angiocardiography.

Sometimes the aorta does not cross to the left as soon as it arches around the right main bronchus but descends to the right of the spinal column to cross lower down so as to enter the aortic hiatus. In such cases, the descending aorta can be seen roentgenologically to be on the right instead of on the left and the aortic knob also will be seen on the right. This is known as right aortic arch with "low-crossing."

In most instances the usual roentgenograms and roentgenoscopy are all that are needed to support the diagnosis of tetralogy of Fallot. In some cases, however, angiocardiography will aid materially. Eisenmenger's complex is similar to the tetralogy of Fallot except that there is no pulmonary stenosis and the conus arteriosus and pulmonary artery are enlarged. Operation is not indicated in cases of Eisenmenger's complex, but angiocardiography might be needed to distinguish this condition from pulmonary stenosis associated with poststenotic dilatation of the pulmonary artery. Transposition of the great vessels and truncus arteriosus could be identified by angiocardiography. Coarctation of the aorta may be found associated with the tetralogy of Fallot. Any abnormality of the aorta and the course of the major branches of the aorta can be shown by angiocardiography and this may be important if operation is contemplated.

Coarctation of the aorta is frequently, an isolated vascular anomaly. At times it is found associated with other cardiovascular anomalies but, as a rule, these are not significant. A patent ductus arteriosus may be associated with coarctation of the aorta. In cases in which the patients are adults, the roentgenologic diagnosis of coarctation of the aorta is usually made with ease since there is notching of the inferior aspects of the posterior portions of the ribs, especially from the third to the ninth. When this is seen, it is pathog-

nomonic. Notching of the ribs is usually not seen in children, however, and, since surgical intervention is desirable before irreversible degenerative cardiovascular changes have taken place, it will be necessary for roentgenologists to look for other evidences of coarctation of the aorta. In coarctation of the aorta hypertrophy of the left ventricle develops sooner or later but this may be caused by many other conditions. The aortic knob is absent or small but this occurs in other conditions, especially in children. The ascending aorta appears normal or is prominent on the right. In the left anterior oblique view it may be possible to see the constricted segment of the aorta. This can be seen most frequently in older patients in whom atheromatous degeneration and calcification of the aorta allow the entire arch and upper part of the descending aorta to be seen roentgenologically. In cases in which the patients are children, the constricted segment can rarely be seen in the roentgenogram. According to Crafoord *et al.*,⁴ the ligamentum arteriosus draws the stenosed segment of the aorta ventrally and medially, and they said that this angulation of the aorta may be seen roentgenologically. According to them, what appears to be an aortic knob may, in reality, be a strongly pulsating enlarged left subclavian artery which has a convex left outline.

The constricted segment of the aorta can be demonstrated by angiocardiography.^{8,18,19} It remains to be seen how

frequently this method will demonstrate the caliber and length of the constriction. This would be of immeasurable aid in deciding whether the segment could be excised. If the caliber of the stenosed segment were known, it would also give some indication as to the degree of collateral circulation that has developed. Angiocardiography can provide some direct evidence as to the collateral circulation since it shows very clearly the size of the great branches of the aorta proximal to the site of coarctation and also shows the size of the internal mammary arteries.^{8,18,19} A good collateral circulation must be established in these cases before operation can be attempted since the aorta is clamped off for a considerable period of time during the operation and the collateral circulation must be adequate to provide for the trunk and lower extremities during that time. Angiocardiography might reveal unsuspected cardiovascular anomalies that would otherwise not be suspected and knowledge of their presence might alter the surgical aspect considerably.

It is hoped that further advances will be made in the surgical treatment of congenital heart disease. It may be found that other types of congenital cardiac abnormality will be amenable to surgical treatment. Careful roentgenologic observations should aid in the making of an accurate diagnosis and angiocardiography especially has much to contribute in the diagnosis of congenital heart disease.

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BOOK REVIEWS AND NOTICES

PROTOZOÖLOGY. By RICHARD R. KUDO, D.Sc., Professor of Zoölogy, University of Illinois. 3rd ed. Pp. 778; 336 ills. Springfield, Ill., Thomas, 1946. Price, \$8.00.

THE forms and adaptation of the Protozoa are hardly more varied than are the relationships of protozoölogy to other branches of biology. Recent events have served to emphasize the potential importance of parasitic Protozoa in human medicine when warfare upsets the artificial barriers which have come to limit these organisms to the less fortunate elements of mankind or to the less favored parts of the earth. Veterinary medicine also knows the potentialities of the protozoan parasites of domesticated mammals and birds. The ichthyologist, the apiculturist and the entomologist also must reckon with protozoan parasites. However, the non-parasitic forms of this group of animals are equally interesting. They serve as material for the cell physiologist, the geneticist, the ecologist; they are concerned in water purification and sewage disposal; the skeletons of certain groups supply the petroleum geologists and paleontologists with a key to the age of sedimentary formations. Professor Kudo presents an excellent and well-balanced discussion of the entire group. The book is intended primarily for advanced students but anyone whose interests require knowledge of or reference to the Protozoa will find it a valuable source of information.

H. R.

THE DOCTOR IN THE FRENCH LITERATURE OF THE SIXTEENTH CENTURY. By NANCY F. OSBORNE. Pp. 140. New York: King's Crown Press, 1946. Price, \$2.00.

PERHAPS because literature tends to give a wrong focusing on the individual, *i. e.*, a caricature, whether intentional or otherwise, the author has most to say about the Renaissance practitioner's "ignorance, avarice, jealousy, chicanery, and arrogance." On the other hand, she gladly recognizes such exceptions to the rule as Rabelais, Fernel, Paré and a number of others. The points

asserted in the text are supported by frequent quotations from contemporary sources, which add considerably to the book's attraction if not too difficult in their old-fashioned French. The apothecary, the charlatan, remedies and their modes of administration receive deserved castigation; yet the author admits an affection for the "pompous old fool" with his jovial humor and spicy wit who typified the French physician of the 16th century. Thence doubtless is derived much of the charm of this interesting production. E. K.

TEXTBOOK OF NEURO-ANATOMY. By ALBERT KUNTZ, PH.D., M.D., Professor of Micro-anatomy, University of Missouri, School of Medicine. 4th ed. Pp. 478; 325 ills. Phila.: Lea & Febiger, 1945. Price, \$3.25.

THOUGH this new edition of a standard medical textbook of neuro-anatomy cannot solve the problem of learning neuro-anatomy, the text has the advantage of being clearly written and has summaries at the end of each chapter which will appeal to the medical student or others interested in review. The illustrations have been improved since the last edition. As might be expected from some of the author's other work, the chapter on the autonomic nervous system is unusually detailed and authoritative.

W. H.

MODERN UROLOGY FOR NURSES. By S. M. DWYER and G. W. FISH. 2nd Ed. Pp. 287. Phila.: Lea & Febiger, 1945. Price, \$3.25.

THIS well-planned and well-written treatise fulfils its objective, which is to give the graduate and student nurse a working knowledge of urologic disease, to better enable her to more intelligently carry out her duties incident to the care of the urologic patient. Its value should be notable as a reference book to those teaching urologic nursing and to those in charge of planning or founding a new urologic department.

There are chapters on floor plan, person-

nel and equipment for a urologic service; use and care of instruments and apparatus; minor urologic procedures; as well as detailed information in regard to preoperative, operative and postoperative care in major urologic conditions.

Each organ of the urinary tract, the kidney, ureter, prostate, bladder, urethra and external genitalia in the male, is discussed as to anatomy, physiology, diseases and treatments. Always the nurses' part in the care of such patients is emphasized. Illustrations are adequate and to the point.

The presentation of this little book should simplify the task of becoming a good urologic nurse for those who will take the time to read it.

L. L.

WAR NEUROSES. By ROY GRINKER, LT. COL., M.C., and JOHN SPIEGEL, MAJ., M.C. Pp. 145. Phila.: Blakiston, 1945. Price, \$2.75.

THIS is one of the more readable and interesting reports on military medicine to come out of the war. Written at the end of the Tunisian Campaign and printed in 1943 for restricted distribution to medical officers, it proved of such merit that with the lifting of military restrictions it is now published for general circulation. Naturally it is primarily of value to the psychiatrist and to all military medical officers. The authors include a description of clinical symptoms, a section of special psychiatric problems of the Air Forces, discussions of etiology, prognosis, treatment and results, concluding with discussion of psycho-biologic dynamics.

While this is a worthwhile contribution to the literature of neurologic disturbances under the special situations of war, it is doubtful whether much of the information gained can be used in handling the usual neuroses encountered in civilian practice. Particularly would this seem to be true of the reactions encountered in narco-synthesis.

W. H.

SECRETORY MECHANISM OF THE DIGESTIVE GLANDS. By B. P. BABKIN. Pp. 900. New York: Hoeber, 1945. Price, \$8.00.

IN the author's own words, "This volume deals with the experimental analysis of the secretory function of the digestive glands in carnivorous animals and man." In it Professor Babkin has undertaken to "sum up

our present knowledge in this province of physiology in order to facilitate further study." As an aid to "further study" this work will serve to orient the prospective investigator in respect to the facts already known, the methods that have been and may be used, the innumerable problems remaining to be studied, and the various interpretations that have been made of previous observations. Originally prepared for use in lectures to graduate students, the material is calculated to stimulate and facilitate research rather than to instruct in the conventional textbook sense.

As interpreted by Professor Babkin, the "mechanism" of secretion includes not only the intimate physico-chemical processes that go on within the secreting cell but also the stimuli which cause the cell to secrete, as well as the origin and mode of transmission of such stimuli, and, finally, the nature and composition of the secreted product. In discussing these problems, the author finds it necessary to consider more than can be learned by physiologic methods alone. The gross and microscopic anatomy of the glands and their associated structures and the chemistry of the secreting cell and its products are also discussed.

As indicated by the title, the book is a dissertation on the secretory mechanism as such rather than an analysis of the function of the individual digestive glands. Consequently, the general arrangement of the subject matter is such as to facilitate the study of the mechanism as a whole. Nevertheless the author has found it convenient to include most of his discussion of the gastric glands in a group of consecutive chapters comprising nearly half the book. Another group of chapters making up about one-third of the book deals with the salivary glands. A third section deals with alimentary hormones. In this section, as elsewhere throughout the book, the nervous regulation of secretion is given adequate recognition. These 3 sections, considered apart from the rest of the material, amount to monographs in their respective fields. The author's warning that his review of the literature is incomplete does not impress the reader who finds here more information than he had hoped ever to find in any one place. The pancreas receives less attention than the other digestive glands, doubtless because the author found it less useful as a means

of illustrating the principles which he chose to discuss.

In this, as in his previous publications, Professor Babkin emphasizes the fact that the digestive glands, particularly the gastric and salivary glands, are complex structures made up of cells of different types, each of which elaborates its own special secretory product. In his view the several types of cells may be stimulated separately by appropriate nervous or humoral stimuli and this fact accounts for the observed variations in composition of the individual digestive secretions.

The book contains an excellent index and a bibliography of more than 1600 references, complete with titles. Many of the latter refer to dissertations in the Russian language which have had only a limited circulation, even in Russia. These will prove most valuable to English speaking students insofar as they are reviewed in the text. J. T.

CLEFT PALATE AND SPEECH. By MURIEL E. MORLEY, B.Sc., F.C.S.T., Speech Therapist to the Royal Victoria Infirmary, the Hospital for Sick Children, and the Newcastle General Hospital, Newcastle-upon-Tyne. Pp. 160; 52 ills. Baltimore: Williams & Wilkins, 1945. Price, \$2.75.

THIS little book is intended primarily as a guide for speech therapists in the training of patients with speech defects due to cleft palate. Chapters 1 and 2 present a very clear concise description of the embryologic, anatomic and physiologic factors involved in cleft palate, and explains fully the defects in speech resulting from the various types. The object of surgical treatment is carefully explained in Chapter 3, with special reference to the methods employed by Wardill, with whom the author is most closely associated. In Chapter 4 is a discussion of feeding and speech problems in cleft palate. An analysis of speech defects before and after operation, with methods of examination, is given in detail in Chapter 5. Chapters 6 and 7 cover treatment by the speech therapist, and Chapter 8 records 25 case histories showing development of speech in various types and conditions.

This is the most complete, and at the same time readily understood and reliable presentation of the problems associated with cleft palate that has come to our atten-

tion. No one, including the surgeon, interested in the problems of cleft palate, can afford to be without the book. R. I.

PERSONALITY IN ARTERIAL HYPERTENSION.

By C. A. L. BINGER, M.D., N. W. ACKERMAN, M.D., A. E. COHN, M.D., H. A. SCHROEDER, M.D., and J. M. STEELE, M.D. Pp. 228. New York: The Am. Soc. for Res. in Psychosomatic Problems, 1945. Price, \$3.00.

THE authors have added another excellent study to the series of Psychosomatic Medicine Monographs. Personality studies together with an evaluation of the environmental situations which produced them in 24 patients with hypertension have been outlined together with interpretative remarks concerning the psychodynamics. It is interesting to note that these personality evaluations show a very consistent pattern despite the varied somatic pathology of the hypertension.

The authors have been conservative in their conclusions concerning the etiologic significance of the psychiatric observations. They conclude that "these cases are characterized by two phenomena, namely, elevation in blood pressure and disturbances in personality," but neither the elevation in blood pressure nor the disturbances in personality imply the existence of the other.

H. G.

EXPERIMENTAL CATATONIA. By HERMAN HOLLAND DE JONG, M.D. Pp. 225. Baltimore: Williams & Wilkins, 1945. Price, \$4.00.

THIS book is a report of further work by the author and his associates upon the experimental production of catatonia. It was early found that many substances besides bulbo-capnine produced this reaction and a search was made for a "catatonizing chemical nucleus." But since even extremely simple substances such as nitrogen could be used to induce these states, it was concluded that the essential element was cellular asphyxiation which operated by changing the threshold of cellular discharge. Since experimental catatonia can be induced by a great variety of chemical and biochemical substances, by alterations of metabolism (for example, ligation of the hepatic artery),

by electrical shocks, by brain lesions, by audiogenic stimuli, etc., it was thought that it represents a general reaction form of the central nervous system. This is strengthened by the fact that work was done with representatives of the many animal species.

Studies were also made of schizophrenics, in a search for specific toxic substances in the urine; but these results have been negative.

Although experimental catatonia is regarded as a general reaction form of the nervous system, a certain specificity emerged in the fact that only the alteration of either liver or intestinal function produced the condition. From this the authors postulate a possible primary liver damage as the basis for certain cases of catatonia and schizophrenia, an hypothesis supported by finding that a high percentage of schizophrenics reacted positively to the cephalin-cholesterol flocculation test as compared with the controls. The book is interestingly and simply written and should be informative and thought provoking for all those interested in this field.

L. S.

SKIN DISEASES IN CHILDREN. By GEORGE M. MACKEE, M.D., and ANTHONY C. CIPOLLARO, M.D. 2nd ed. Pp. 436; 225 ills. New York: Hoeber, 1946. Price, \$7.50.

THE senior author of this book on pediatric dermatology, the 1st edition of which appeared in 1936, is Professor of Clinical Dermatology and Syphilology at the New York Post-Graduate Medical School of Columbia University; the junior author is an Associate in Dermatology and Syphilology at the same institution. There are chapters by Herman Beerman on syphilis in children, by Frances Pascher on allergic dermatoses in children, by Eugene Traub on congenital cutaneous anomalies, and by Nathan Lobel on contagious diseases.

As skin diseases of infancy and childhood are of great importance to all physicians, and in general are not covered adequately in standard textbooks on dermatology, a new enlarged edition of this practical book is welcomed.

The authors' aim has been to make this treatise comprehensive, with emphasis on etiology and treatment. In order to simplify the presentation of material, they have grouped various diseases on the basis of

etiology, pathology or clinical similarity. In general, this classification is a workable one. However, there are instances in which it leads to some confusion in regard to etiology of certain dermatoses. For example, the authors have included *dermatitis repens* and *pompholyx (dysidrosis)* under diseases due to fungi. Few dermatologists would agree with this classification.

The section on benign and malignant new growths, congenital cutaneous anomalies, diseases of the sweat glands, sebaceous glands, hair and nails, the tuberculosis group of skin diseases, and syphilis in children are of particular value because the authors have so summarized their wide experience that the physician can find authoritative answers to many of his problems without difficulty. For example, the discussion on what to do and what not to do about nevi is excellent.

In some instances, the lack of critical presentation of material is regrettable. For example, an entire page is devoted to the low fat diet method of treating acne vulgaris, without any mention of the authors' personal opinion. It is difficult to accept the statement on page 16 that "impetigo contagiosa is usually caused by a streptococcus and rarely by the *Staphylococcus aureus*" in the light of the reports of many investigators to the contrary. If the authors had had wider experience in the use of penicillin topically in the treatment of pyogenic infections of the skin, they would probably not have stated that penicillin ointment gives "spectacular" results in sycosis vulgaris.

It would seem that in a book intended chiefly for the general practitioner, it would have been preferable to have outlined methods of treatment which have proved to be effective in their hands, with a definite statement of their personal opinions on the efficacy of method.

Despite these shortcomings, this is a useful book, and the authors are to be congratulated on bringing together a great amount of valuable information, and presenting it in a concise manner.

D. P.

MEDICAL CLINICS OF NORTH AMERICA. *Problems in Postwar Medicine.* March 1946. From the 2nd Service Command. Pp. 243-485. Phila.: Saunders, 1946. Price, \$16.00 a year.

THIS is a well-written symposium which stands as a tribute to its various authors,

who, in spite of the fact that they were pressed by the needs of war, found time to carry out these original pieces of clinical research. In it they accomplish 2 things: First, an illustration of the strides made in the treatment of various conditions, commonly met by both military and civilian physicians. The second is the fact that the articles acquaint the civilian physician with the problems and treatment of certain disease entities which occurred in military personnel, so that the personnel may be better following their discharge.

Of special interest are the timely and practical articles on the "Problem of Nutrition in the Treatment of the Prolonged Hospitalized Patient" and the "Significance of a Psychiatric Diagnosis." A. C., Jr.

NEW EDITIONS

Physiological Chemistry. By J. F. McCLENDON, PH.D. 7th ed. Pp. 463; 83 ills. St. Louis: Mosby, 1946. Price, \$4.25.

NEW BOOKS

Three Unpublished Drawings of the Anatomy of the Human Ear. By the late MAX BRÖDEL. Assisted by P. D. MALONE, STACY R. GUILD and S. J. CROWE. Phila.: Saunders, 1946.

In addition to the drawings there is an attractive color reproduction of a portrait of Mr. Brödel.

William Beaumont's Formative Years. Two Early Notebooks 1811-1821. With Annotations and an Introductory Essay by GENEVIEVE MILLER, M.A., Institute of the History of Medicine, The Johns Hopkins University. Pp. 87; 21 ills. New York: Schuman, 1946. Price, \$5.00.

AN attractive presentation by Genevieve Miller of the notebooks of Beaumont that were begun by him while he was studying with Dr. Benjamin Chandler in Vermont.

NOTICE AND INSTRUCTIONS TO CONTRIBUTORS

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ORIGINAL ARTICLES

THE EFFECT OF DI-ISOPROPYL-FLUOROPHOSPHATE (DFP) UPON PATIENTS WITH MYASTHENIA GRAVIS*

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DI-ISOPROPYL-FLUOROPHOSPHATE (DFP) is a compound (completely unrelated chemically to neostigmine or physostigmine) which has been shown to produce marked inhibition of serum, red cell, muscle and brain cholinesterase for long periods of time. The rates of restoration of these activities are very slow following administration of DFP and consequently the effects of DFP are far more prolonged than those of neostigmine or physostigmine.^{9,10} When instilled into the conjunctival sac, 0.1% DFP produces intense miosis which may persist for days or weeks;¹¹ this action has been found to be of considerable value in the treatment of glaucoma and in overcoming atropine mydriasis.¹² When given systemically in small doses, its only detectable effects are

the inhibition of plasma and then red cell cholinesterase; large doses produced stimulant effects upon skeletal muscle, and still larger doses lead to increased gastro-intestinal activity and (in some patients) effects upon the central nervous system. Because of its pronounced and prolonged anticholinesterase action, one of us (A. G.) considered it desirable to evaluate its effects upon patients with myasthenia gravis. Since many investigators believe that the symptoms of myasthenia gravis are due to an insufficiency of acetylcholine at the myoneural junction,[†] it seemed a matter of great theoretical importance to determine whether this new anticholinesterase agent was capable of increasing muscle strength in myasthenic patients. If DFP could be shown to possess this

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† This insufficiency may be due to decreased formation of acetylcholine (cholinergic defect), to increased cholinesterase activity at the myoneural junction, or to decreased activity of acetylcholine due to the presence of antagonistic circulating curare-like substances.

property, its prolonged action would be important clinically by reducing the number of doses of drug needed and by providing a relatively constant effect throughout its period of action.

Method. In order to evaluate the efficacy of DFP, both subjective improvement noted by patients and observers and objective tests were employed.¹⁹ Whenever possible, a comparison was made of the effects of DFP and neostigmine.

The effects of DFP or neostigmine upon muscle weakness, ptosis, swallowing function, appetite and hand dynamometer readings were noted by each patient and by one observer (J. T.) 3 times daily throughout the investigation. In addition, certain tests were performed in duplicate daily by each patient. These consisted of:

(a) *A Dynamic Finger Test.* The left arm was firmly enclosed in a plaster cast from above the elbow to the proximal phalanges to prevent movement of any muscles except those of the left forefinger. A light metal splint was then fastened with adhesive tape to the back of the forefinger to prevent flexion at any joint except at the phalangeal metacarpal. To the middle phalanx was attached a cloth ring, $\frac{1}{2}$ inch wide; strong twine led from this to a pulley and a standard weight of 840 gm. The weight could thus be pulled up and down (on guide bars) by the finger and the rate and extent of this movement was recorded on kymograph paper by an ink writer attached to the weight. The maximal amplitude through which the finger could be flexed and extended passively was recorded before each test. A rate of 60 complete pulls per minute was then imposed upon the subject by a metronome. The subject was instructed to pull as vigorously as possible at this rate for 30 seconds.

(b) *A Static Finger Test.* The same apparatus (splints and weight) was used, but in this test the finger was pulled forward to an angle of 90° and the subject was instructed to hold the finger there with weight attached, as long as possible. Typical records of the dynamic and static

tests are shown in Figure 1. In both the dynamic and static tests a rectangular area enclosing the maximal possible contraction for 10 seconds was measured by a planimeter for each test and this was considered as 100%; the area on the record enclosed in each 10 second portion of the 30 second test was measured similarly and the per cent of maximum is indicated as static or dynamic tests 1, 2 and 3 in Tables 2, 4 and 5. A normal individual can perform for many minutes without any decrease in performance, but myasthenic patients generally tire within 10 seconds.

(c) *Maximal Breathing Capacity.*⁵ This was employed in some subjects as a dynamic vital capacity test¹² for measurement of strength of the respiratory muscles. The individual was instructed to breathe as rapidly and as deeply as possible for 30 seconds; the expired air was collected in a spirometer and the volume was measured for the first and second 15 second periods separately.

(d) *Hand dynamometer tests* were performed 3 times daily.

Patient L. G. had marked right monocular myasthenia gravis. In this case, in addition to the above tests, special ocular measurements were made so that objective comparison might be made of the effects of neostigmine and DFP on the extraocular muscles. Ocular muscle movements were measured by the screen and parallax method. The patient's head was placed in the head-rest of a Zeiss perimeter. The right eye was centered on the central fixation point when the right field of rotations was tested. The left eye was centered on testing the left field of rotations. The light of fixation was placed 30° from fixation in the 6 cardinal directions of gaze. By using the perimeter for measurements, some of the errors usually encountered in the estimation of squints and phorias were decidedly lessened. The distance from the eye to the point of fixation was constant in the 6 cardinal directions, and could be duplicated. The prisms were held approximately 2 cm.

from the eye and careful attention was paid to the position in which the prisms were held. Attempts were thus made to avoid the errors inherent in this method of measurement as described by Putnam and Quereau.¹⁷

In most of the subjects, mechanical myograms and electromyograms were recorded following supramaximal stimulation of the ulnar nerve; recordings were made from the fifth finger.⁶ Rates of stimulation were 5, 20, 30, 40 and 55 per second.

ity, or indeed of enzyme activity in general, after therapy requires close scrutiny. When the substance which forms a readily reversible compound with cholinesterase and thus inhibits its activity, is administered therapeutically, the observed cholinesterase activity of the drawn serum or red cells depends upon the degree of dissociation of the cholinesterase inhibitor complex. This in turn depends, among other factors, on the dilution of serum or red cells in the final reaction mixture. Straus and Goldstein²⁰ have studied in

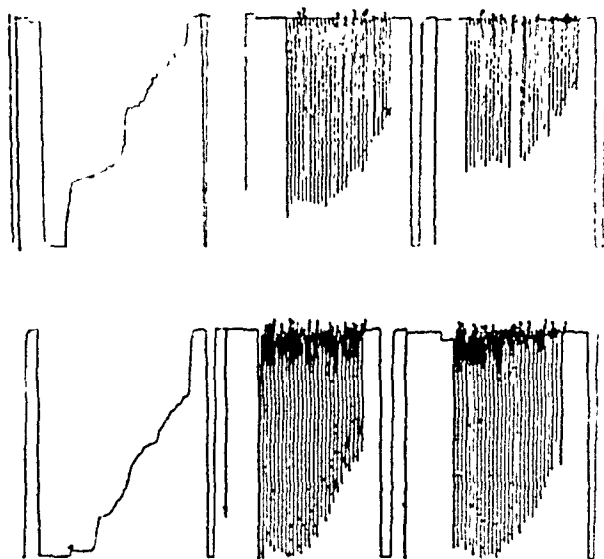


FIG. 1.—Static (left) and dynamic (center and right) work tests in Patient 6 before DFP (upper record) and 12 hours after the injection of 3 mg. of DFP (lower record).

The effect of DFP upon the function of the liver, kidneys, heart, hematopoietic systems and upon other body functions was evaluated carefully in each patient; these studies were essentially negative and have been reported elsewhere.⁴

Cholinesterase determinations were done before and during DFP therapy by a modification of Ammon's method.¹⁶ It is of interest to note that plasma and red cell cholinesterase were within the normal range in all of our untreated myasthenic patients. In Table 1 are shown the physical characteristics of the 7 patients, the treatment course employed, and the lowest cholinesterase levels attained during DFP therapy. The interpretation of the measurement of cholinesterase activity,

detail the inhibition of dog and horse serum cholinesterase by physostigmine and have shown how the cholinesterase activity of the serum at any dilution may be corrected so as to represent the cholinesterase activity of the undiluted serum *in vivo*.

Neostigmine and other compounds have also been shown to form reversible complexes with cholinesterases.² However, no study of the effect of neostigmine on cholinesterase, similar to that of Straus and Goldstein on physostigmine, has yet been made. A number of determinations of cholinesterase activity in our patients showed, as might have been expected, that after the administration of neostigmine, the enzyme activity varied with

the dilution of the enzyme and the stage of the reaction. In view of these considerations and since we were not in a position to evaluate comprehensively the effect of neostigmine at various enzyme concentrations, we have not recorded any cholinesterase activities after neostigmine administration.

was possible in this patient. She required no further medication for 3 weeks, during which time her plasma cholinesterase activity rose from 2 to 122% and red cell cholinesterase from 37 to 50% of the control values. The myasthenic symptoms then reappeared. To evaluate possible psychic components in this patient's symptomatology she was given injections of sterile saline solution.

TABLE 1.—SUMMARY OF DFP THERAPY IN PATIENTS WITH MYASTHENIA GRAVIS

Patient	Sex	Age	Wt. lbs.	Total mg. DFP		DFP treatment period (days)	Period of observation post-treat- ment (days)	Lowest cholinesterase activity during DFP therapy		Remark
				I.M.	Orally			Plasma (%)	R.B.C. (%)	
1. H. G.	F	25	148	11.0	..	29	295	2	24	Myasthenia gravis
2. S. C.	F	29	128	25.6	..	32	85	2	15	Myasthenia gravis
3. L. G.	M	54	131	17.0	168	117	67	0	26	Ocular myasthenia gravis
4. R. G.	F	21	107	3.0	28	8	38	5	74	Severe myasthenia gravis; patient requested release from hospital; died 6 months later
				2.5	..	1	5			
5. R. W.	F	25	90	5.0	92	26	11	0	25	Severe myasthenia gravis; died at home 1 month later
6. A. D.	F	38	93	13.0	60	50	30	0	5	Severe myasthenia gravis; died at home 1 month later
7. N. A.	F	35	94	5.5	12	6	3	1	18	Old myasthenia gravis

In contrast, Mazur and Bodansky showed that the inhibition of human serum by DFP is not readily reversible and that dilution of serum from a DFP injected animal does not influence the relative activity of cholinesterase on dilution of the serum.¹⁶ These observations have been amply confirmed in the present study and accordingly cholinesterase determinations after DFP administration have been used as measures of the enzyme activity *in vivo* following DFP administration.

Results. *Patient 1* (H. G.) had myasthenia gravis of 4 years duration. Her outstanding symptoms were difficulty in swallowing and speaking, and weakness of arms, legs, neck and eyelids. Previous therapy had included irradiation over the region of the thymus gland and neostigmine. Electromyographic studies coupled with characteristic neostigmine, quinine and erythroidine tests confirmed the diagnosis of myasthenia gravis. This patient showed a dramatic improvement after the administration of 7 mg. of DFP intramuscularly over a period of 5 days, as judged by subjective symptoms, general appearance and hand dynamometer performances; no objective quantitative comparison with neostigmine

Since this procedure resulted in almost complete return of strength, the patient was no longer studied.

Patient 2 (S. C.) had developed ptosis of her lids, double vision, difficulty in speech and swallowing, and limb weakness 4 years before this study. For the past 3 years she had been taking 15 mg. of neostigmine bromide 6 to 8 times daily as her sole form of therapy; under this régime she had been able to continue in her secretarial position. The electromyograph, erythroidine and intocostarin (curare) tests, and the static and dynamic finger tests revealed a typical picture of myasthenia gravis. This patient was given a prolonged course of intramuscular and oral DFP therapy both as an in and out patient. As a rule, marked subjective and objective improvement followed its use. Of the hundreds of tests done upon this patient, several representative results are shown in Table 2. The static and dynamic finger tests indicated that the associated muscles improved upon DFP therapy almost to the same degree as during neostigmine treatment (Table 2). During DFP therapy, she was able to lead a normal life in the hospital without neostigmine for 2 weeks, though she had formerly required daily amounts of 120 mg. neostigmine bromide orally. However, when she returned to her position as

secretary and typist, she was unable to perform a full day's work without additional neostigmine. During a 3 week period, she received 1 or 2 injections a week of 2 to 3 mg. DFP intramuscularly. On the day of the injection she required little or no neostigmine, but within 1 to 2 days, 40 to 60 mg. daily were needed. Attempts to increase the dose of DFP intramuscularly led to severe nausea and vomiting. Later she was given daily oral doses of DFP, and this served to reduce the amount of neostigmine needed. This patient requested prolonged treatment with DFP because of

visual blurring completely. Physical examination revealed ptosis of the right upper eyelid and weakness of the right inferior oblique and right inferior rectus muscles. The left eye was normal.

This patient had myasthenia gravis limited to the right eye since muscle function tests done upon the hands and fingers revealed no weakness even with prolonged testing. Consequently the comparison of the effectiveness of DFP and neostigmine was carried out entirely upon the extraocular muscles. Measurements were made before and 30, 35, 45 minutes and 6 hours after injection

TABLE 2.—EFFECT OF NEOSTIGMINE AND DFP UPON MUSCLE FUNCTION TESTS IN PATIENT 2 (S. C.)

				% of possible maximum by 10 second periods								
Date	Drug	Dose (mg.)	Hours after drug test was performed	Static test			Dynamic test			Dyna- mometer		R.B.C. Ch. E.
				1	2	3	1	2	3	R.	L.	
4/25/45	Neostigmine	1.5 (i.m.)	1½	97	97	91	98	96	88	55	64	*
4/27/45	DFP	3.0 (i.m.)	7	86	87	63	91	90	73	51	50	33%
5/3/45	DFP	3.0 (i.m.)	13	97	100	89	96	90		40	45	22%
				99	100	84	99	92				

* See under Methods for explanation of omission of cholinesterase following neostigmine

TABLE 3.—EFFECT OF NEOSTIGMINE AND DFP ON MYASTHENIC EXTRAOCULAR MUSCLES IN PATIENT 3 (L. G.)

Direction of gaze	Without medication				After prostigmine (1.5 mg.)				After DFP (3 mg.)	
	July 2	July 6	July 9	July 12	July 2 (30 min.)	July 9 (15 min.)	July 12 (35 min.)	July 12 (6 hrs.)	July 6 (15 min.)	July 7 (2½ hrs.)
					Prism diopters					
Up and to right	3†	0	0	0	1†	0	3†	0	0	0
Horizontally and to right	1*	1*	1*	1*	0	1*	3*	1*	0	0
Down and to right	20*	15*	20*	15*	15*	15*	15*	20*	15*	15*
Up and to left	25†	25†	25†	25†	15†	15†	20†	25†	25†	25†
Horizontally and to left	8†	8†	12†	8†	5†	0	1†	3†	10†	10†
Down and to left	0	1†	0	2†	0	0	0	0	0	3*
Fissure size OD (mm.)	2	3	3	3	10	8	8	3	5	6
Fissure size OS (mm.)	10.5	10	11	9	10.5	9.5	9	10	9.5	9

* Right hyperphoria.

† Left hyperphoria.

the overnight effect of the drug; she felt much less weak upon awakening each morning than when maintained on neostigmine alone. In this patient, DFP definitely reduced the simultaneous neostigmine requirement, but could not replace it entirely because of the development of nausea and vomiting (not always controlled by atropine).

Patient 3 (L. G.) had complete ptosis of his right eyelid associated with marked visual blurring when this lid was propped open. Previous therapy had consisted of the administration of 15 mg. of neostigmine bromide 6 times daily which relieved this ptosis in large part but did not relieve his

of 1.6 mg. neostigmine on each of 3 occasions. On different days, measurements were made before and after the intramuscular injection of 3 mg. of DFP. In addition the width of the palpebral fissures was measured before and after each injection. The results are recorded in Table 3.

It is evident that neostigmine had only a slight effect upon the myasthenic extraocular muscles, but a definite effect upon the levator palpebrarum. DFP had less effect than neostigmine on the extraocular muscles and the levator despite the fact that large enough amounts of DFP were given to cause repeated nausea and vomit-

ing. Though less powerful, the DFP action was more prolonged, and some effects persisted for 24 hours whereas that of neostigmine was gone within 6 hours. It is of interest to note that local administration of neostigmine (by iontophoresis of a 5% solution through the right lid) produced definite improvement locally for less than 6 hours.

Patient 3 (R. G.) had marked muscular weakness throughout her body for the past 2 years. She had great difficulty in speaking and swallowing and on several occasions had experienced attacks of dyspnea and tightness in the chest. Her therapy has consisted of 6 to 7 hypodermic injections of 0.5 mg. of neostigmine methyl sulfate and two 15 mg. oral tablets of neostigmine bromide daily. The electromyogram, static and dynamic finger tests, and maximal

to 36 hours. The patient refused further treatment; she died at home 6 months later.

Patient 5 (R. W.) had severe myasthenia of 7 years duration. In the past 2 months she had several periods of respiratory failure and asphyxia. She had been taking 15 mg. of neostigmine orally every 3 hours during the day and every 6 hours at night. Recently she failed to get relief from even 30 mg. of neostigmine orally and hypodermic injections have been required several times daily. The electromyogram, static and dynamic finger tests and hand dynamometer tests were all confirmatory of the diagnosis; these returned toward normal following an injection of neostigmine.

A comparison of the effects of neostigmine and DFP upon this patient are shown in Table 4. Myasthenic symptoms were so severe in this patient that control studies

TABLE 4.—EFFECT OF NEOSTIGMINE AND DFP UPON MUSCLE FUNCTION TESTS IN PATIENT 5 (R. W.)

Date 1945	Drug	Dose (mg.)	Hours after drug tests were performed	% of possible maximum by 10 second periods									Maximal breathing capacity		Dyna- mometer		R.B.C. cholin- ase
				Static test			Dynamic test										
				1	2	3	1	2	3	1	2	R.	L.				
6/27	Neostigm.	15 oral	6	9	9	5	15	12	8	5	13	0	0				
6/23	Neostigm.	1.0 i.m.	$\frac{1}{2}$	93	86	62	79	71	56	92	87	45	40				
6/26	Neostigm.	1.5 i.m.	$\frac{1}{2}$	90	90	90	99	100	72	45	35				
6/25	Neostigm.	1.0 i.m.	$\frac{1}{2}$	95	95	97	97	80	..	77	95	45	32				
6/28	DFP	3.0 i.m.	23	9	19	..	14	20	..	80	59	10	0	60%			
6/29	DFP	2.0 i.m.	1 $\frac{1}{2}$	62	59	53	47	48	..	106	84	20	10	40%			
7/19	DFP	4 oral	2	45	43	43	57	56	..	81	67	20	0	25%			

breathing capacity were all indicative of severe myasthenia gravis and all of these improved markedly following the injection of 1.5 mg. of neostigmine methyl sulfate.

After an injection of 2.5 mg. DFP (in water) intramuscularly, she became nauseated and vomited several times. She complained of dizziness, light headedness and faintness. Attempts to obtain objective measurements of strength of her finger or respiratory muscles failed because of weakness, malaise and inability to sit in a chair. Dynamometer readings were 0. She was given neostigmine every 2 hours again and after 36 hours had regained her previous state of strength.

In this patient, incapacitating side effects of DFP in small dosage prevented an adequate therapeutic trial with this agent. It was apparent, however, that it was unable to substitute for neostigmine even for 4 hours, and that its side effects lasted 24

could not be performed with all drugs discontinued. However, upon 1 occasion, neostigmine was withdrawn for a period of 6 hours; this led to extreme weakness. As shown in Line 1 of Table 4 the static finger test at this time was only 9% of the maximal possible contraction, the dynamic test was 15% and the dynamometer reading was 0. The next 4 lines of Table 4 show the values recorded upon 4 different days 15 to 60 minutes after the injection of 1 and 1.75 mg. of neostigmine intramuscularly; marked improvement is evident. The last 3 lines present the values recorded following the administration of DFP; in each of these no neostigmine had been given for 3 to 4 hours. It can be seen that, while DFP produced considerable improvement in all the tests as compared with the control values, it never improved the patient's static, dynamic and dynamometer scores to the extent that neostigmine did. However, the

respiratory muscles were maintained in relatively good strength by DFP, despite a more marked decline in the second of the two 15 second periods.

This patient was highly coöperative and intelligent and it is felt that she performed to the best of her ability at all times. In this case, DFP was unable to substitute even in part for neostigmine (see discussion). This patient was returned to neostigmine therapy, but died in her sleep at home a few weeks later.

DFP given alone (even to the extent of lowering red cell cholinesterase to 5% and of producing nausea and vomiting) was never able to raise the scores to the high levels attained following the addition of neostigmine. This table indicates that combined therapy of DFP and neostigmine may yield more improvement than either drug alone. This patient died at home 1 month later while on neostigmine therapy.

One experiment upon this patient raises some pertinent questions regarding the

TABLE 5.—EFFECT OF NEOSTIGMINE AND DFP UPON MUSCLE FUNCTION TESTS IN PATIENT 6 (A. D.)

Date 1945	Drug	Dose (mg.)	Hours after drug tests were performed	% of possible maximum by 10 second periods									Maximal breathing capacity		Dyna- mometer		R.B.C. cholin- erase
				Static test			Dynamic test										
				1	2	3	1	2	3	1	2	R.	L.				
4/23	Neostigm.	15 oral	4	87	70	56	82	70	55	45	35				
5/3	Neostigm.	1.5 i.m.	2	94	94	92	92	83	39	78	56	50	50				
5/3	Neostigm.	1.5 i.m.	2½	100	100	87	89	84	72	40	45				
5/9	Neostigm.	15 oral	1	96	85	87	91	73	40	45				
			1½	100	100	73	98	79	40	45				
5/10	Neostigm.	15 oral	2	93	99	100	96	100	65	40	45				
				98	100	91	100	99	40	45				
5/12	Neostigm.	15 oral	3	100	100	100	99	100	89	45	42				
				90	91	93	96	93	80	45	42				
4/24	DFP	3.0 i.m.	2½	87	66	..	72	60	..	45	38	45	38	70%			
			4½	79	51	..	69	59	26	30	30	61%			
4/29	DFP	2.0 i.m.	19	73	62	48	79	63	30	30				
			26	64	58	34	84	78	30	30				
5/6	DFP	3.0 i.m.	26	93	75	48	100	78	30	32	25%			
			26½	93	67	..	87	82	30	32				

Patient 6 (A. D.) had double vision, difficulty in swallowing and talking, and generalized weakness. She had never had neostigmine therapy. Her myographic, electromyographic, static and dynamic finger, hand dynamometer and maximal breathing capacity tests were all indicative of myasthenia gravis. Intocostin (curare) and erythroidine resulted in marked weakness in one-tenth the dose required to produce lid ptosis in normal individuals and her symptoms and tests improved greatly after an injection of 1.5 mg. neostigmine methyl sulfate.

In Table 5 are shown the scores attained after neostigmine on each of 6 days and those achieved after DFP had been administered alone on each of 3 days. With the exception of that given on April 23, 1945, all doses of neostigmine were administered after some DFP had been injected and the plasma and red cell cholinesterase were already at a low level. It can be seen that the administration of DFP was followed by scores as high as that produced by oral neostigmine on April 23. Figure 1 illustrates the improvement shown in her work tests after DFP. However,

mechanisms of action of DFP and neostigmine in the treatment of myasthenia gravis. After treatment with DFP for a month, the plasma cholinesterase was 0 and the red cell cholinesterase was 14% of normal; nevertheless the patient was extremely weak (see Table 6 and Fig. 2). Neostigmine administered at this time improved her strength markedly. DFP administered 5½ hours later (after the neostigmine effect had apparently worn off) failed to produce further improvement. It has already been pointed out that DFP may be ineffectual if given after neostigmine¹⁰ and this may account for its failure at this time. The reverse is not true, however, for neostigmine produced a considerable return of function at a time when the patient had been administered full doses of DFP, and the plasma and red cell cholinesterase were very low. This indicates either that blood cholinesterase levels are no index of muscle cholinesterase or that neostigmine acts by some mechanism other than by inactivating cholinesterase. This point is discussed later.

Patient 7 (N. A.) had developed weakness of her arms, legs and eyelids and difficulty in chewing and swallowing 14 years previously. Her disease has not progressed for 2 years. An injection of a small amount of erythroidine aggravated her weakness. Her electromyogram was atypical in that the first 3 action potentials of each series increased following supramaximal repetitive nerve stimulation (similar to the process of facilitation described by Harvey *et al.*⁷ in his patient B. W.). The static and dynamic

finger tests and the hand dynamometer test revealed marked muscle weakness. However only slight improvement followed the administration of neostigmine. This patient could not be classified at this time as a typical case of myasthenia gravis. She was given DFP only in the hope that some unexpected improvement might result. Subjective and objective studies showed no improvement or deterioration in her condition following DFP, except for occurrence of more side effects with the latter drug.

TABLE 6.—(COMPARISON OF EFFECT OF DI-ISOPROPYL FLUOROPHOSPHATE AND NEOSTIGMINE ON MUSCLE STRENGTH AND BLOOD CHOLINESTERASE)

Drug	Time	Cholinesterase (%)		% of possible maximum by 10 second periods						Maximum breathing capacity
				Static test			Dynamic test			
		Plasma	R.B.C.	1	2	3	1	2	3	
Previous DFP	9-10 A.M.	0	14	54	47	25	58	55	..	41
Neostigmine, 1 mg. i.m. . .	11:03 A.M.	92	88	89	74	60	44	52
	11:45 to 11:50 A.M.
	1:04 P.M.	2	12
	1:15 to 2:00 P.M.	86	87	86	60	46	..	51
	4:30 P.M.	65	43	..	42	34
DFP, 4 mg. oral	4:35 P.M.
	10:00 to 10:15 P.M.	48	39	13	38	23	..	41

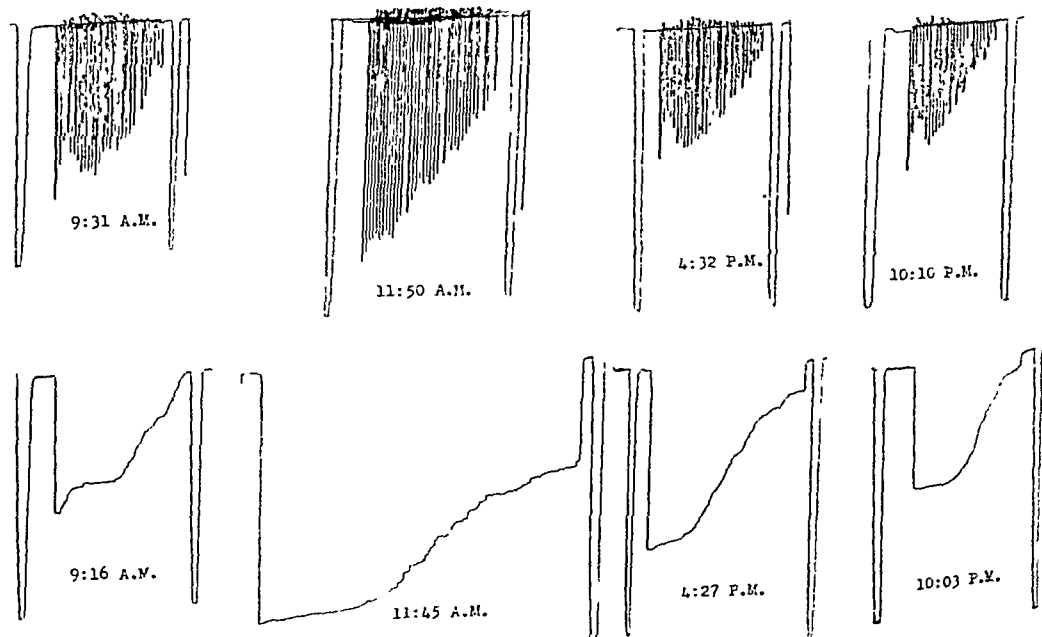


FIG. 2.—Dynamic (upper records) and static (lower records) work tests on Patient 6. The first pair (upper and lower) was recorded at 9:30 A.M. At 11 A.M., 1 mg. neostigmine was injected intramuscularly; the second pair of records was recorded 45 to 60 minutes later. The third pair was recorded at 4:30 P.M., when the neostigmine effect had largely worn off. Immediately thereafter 4 mg. DFP was given orally and the last pair was recorded at 10 P.M. For further discussion, see text.

Discussion. It has been shown by these studies that DFP, an anticholinesterase agent entirely unrelated chemically to neostigmine or physostigmine, is capable of increasing muscle strength in some myasthenic patients. Since the only detectable effects of DFP administered in low dosage appear to be due to its anticholinesterase activity,^{4,16} this reemphasizes that the fundamental defect in myasthenia gravis is intimately related to a deficiency of acetyl choline and that its symptomatic treatment is likewise related to the maintenance of adequate acetylcholine levels at the myoneural region. This "deficiency" of acetylcholine may be due to inadequate synthesis of acetylcholine or to the presence of an antagonistic curare-like substance; this investigation contributes nothing to strengthen or weaken either of these concepts.

However, it does raise the question as to the mechanism of action of neostigmine; for ample subjective and objective evidence has been presented here to show that DFP does not restore muscle power in myasthenic patients to the same degree as does neostigmine. These observations have been confirmed by Harvey and co-workers⁸ who found that intraarterial injections of DFP into localized areas did not produce the *full* improvement in strength and in muscle action potentials that neostigmine did. The question arises as to why DFP, which is capable of lowering plasma and red cell cholinesterase activity to zero, affords less symptomatic relief to myasthenic patients than does neostigmine (the decreases in cholinesterase activity following neostigmine, though not determined precisely, were apparently not of this magnitude). Several explanations may be advanced for this unexpected finding. First, it is possible that the distribution of these compounds in the body is greatly influenced by their physicochemical properties. DFP is highly lipoid soluble whereas neostigmine has a high aqueous solubility. Thus, DFP may readily gain access to nervous tissue in experimental animals,^{7,17} and normal subjects

and myasthenic patients receiving DFP may show evidence of central nervous effects, an action not shared by neostigmine in ordinary doses.⁴ It is possible that neostigmine may have a negligible distribution in the central nervous system whereas it might readily gain intimate contact with the myoneural junction. In support of the view that the relative therapeutic efficacies of DFP and neostigmine are the result of differences in drug distribution throughout the body are the observations of Harvey *et al.*⁸ When large amounts of DFP were injected into the brachial artery of myasthenic patients, muscle strength and muscle action potentials were improved far more than by systemic administration of the drug. Thus it may be assumed that when a sufficient amount of DFP gains access to the myoneural junction, muscle strength is improved almost but not entirely to the same extent as by neostigmine. Unfortunately, doses of DFP large enough to produce such improvement throughout the body cannot be given systemically because of the untoward effects of DFP in large doses upon the gastro-intestinal tract and nervous system.

A second explanation might be offered for the finding that neostigmine is more potent than DFP in the relief of myasthenic symptoms. Neostigmine may have in addition to its anticholinesterase properties a direct action upon skeletal muscle that is not shared by DFP. Few unequivocal experiments have been performed to settle the question of whether direct muscular effects are produced by anticholinesterase agents. So far as their effects upon the circular muscle of the iris is concerned, it has been shown by Anderson for physostigmine¹ and more recently by Leopold and Comroe for neostigmine,¹¹ and for DFP¹² that these drugs become incapable of producing pupillary constriction when the iris is cut off from tonic parasympathetic impulses by ciliary ganglionectomy. So far as skeletal muscle is concerned, Langley and Kato¹¹ were unable to detect any contraction of de-

nervated gastrocnemius muscles following the application of physostigmine. However, Brown and Harvey³ noted that physostigmine produced increased contraction of the denervated *extraocular* muscles, though this effect was not noted when other skeletal muscles were employed. More recently Riker and co-workers¹⁸ stated that they have demonstrated a direct action of neostigmine upon striated muscle fibers. Clearly further quantitative comparisons are needed of the direct effects of physostigmine, neostigmine and DFP upon skeletal muscle.

The fallacy of relating the effects of an anticholinesterase agent upon plasma cholinesterase to its pharmacodynamic actions has already been pointed out.^{9,16} The above studies show that it is equally fallacious to judge therapeutic efficacy by this criterion. Human plasma cholinesterase is more highly susceptible to inactivation by DFP than is human brain or muscle cholinesterase.^{9,16} Thus, even neglecting the added complications of bodily distribution, plasma cholinesterase would be largely inhibited before tissue cholinesterases are significantly affected. Moreover, cholinesterases in different tissues vary so greatly in their rates of regeneration following inactivation by DFP^{9,16} that the enzymatic activity in one tissue cannot possibly be estimated from the analysis of another even though their susceptibilities to inactivation are similar. Thus, the measurement of the cholinesterase activity of erythrocytes affords no knowledge of the status of muscle or brain cholinesterase when DFP is given over a period of days even though the sensitivities of the enzymes at these varied sites fall in the same range.

It has been shown that the DFP-cholinesterase complex is not readily reversible and dilution has no effect upon this reaction. We have already pointed out the uncertainties in using the determinations of cholinesterase activity after neostigmine therapy; the *in vitro* analysis of cholinesterase values in blood taken following neostigmine administration may

yield figures that are considerably above or below the actual enzyme activity in the body, depending upon numerous factors such as dilution, pH and time.

The evaluation of DFP as a therapeutic agent in severe myasthenics is complicated by the fact that neostigmine protects cholinesterase from inactivation by DFP. Koster¹⁰ first demonstrated that animals which had received physostigmine were more resistant to the lethal actions of DFP. Leopold and Comroe¹⁵ have shown that previous ocular administration of physostigmine prevented the characteristic prolonged intense miotic action of DFP, administered at the time of maximal neostigmine miosis. Our Patient 5 (R. W.) could not be deprived of neostigmine for more than a few hours and it is possible that the presence of this reversible inhibitor blocked the effects of subsequently administered DFP. This fact may have some bearing on the failure of DFP in patients with myasthenic symptoms so severe that neostigmine can never be discontinued. Harvey and co-workers have confirmed this by demonstrating that the intraarterial injection of DFP failed to produce its characteristic prolonged effect in patients who had received neostigmine a short time previously.⁸

From the practical point of view, DFP possesses one main advantage, namely its prolonged action. This may enable a patient to avoid marked fluctuations in strength during the daytime and to awaken in the morning with considerably less fatigue than if the short-acting neostigmine alone is used. In our experience, simultaneous use of DFP and neostigmine often resulted in a definite reduction of neostigmine dosage and this combination may prove to be useful in the treatment of myasthenia gravis. However, in view of the marked gastro-intestinal disturbances produced by DFP it is obvious that a compound with a more specific action on the myoneural junction is desirable. Such an agent may be found among the many possible congeners of DFP which are under investigation.

It has been our experience from this study that an evaluation of the effectiveness of drugs in this disease can only be made after considerable experience in the design and interpretation of objective tests has been acquired. Spontaneous remissions in myasthenia are too frequent and unpredictable to permit assay by the patient's subjective impressions alone. Patients with myasthenia gravis want desperately to get well and the psychic effect of any new measure (medical or surgical) may be tremendous.

Conclusions. 1. Di - isopropyl - fluoro-phosphate (DFP), though entirely unrelated chemically to neostigmine, is capable of lowering plasma and red blood cell cholinesterase activity in man to a marked extent and for prolonged periods, and of improving muscle strength in patients with myasthenia gravis.

2. DFP was used in the treatment of 7 patients with myasthenia gravis. Two patients received little or no benefit from DFP, 2 received considerable improve-

ment, 1 received marked beneficial effects and in the other 2 the effects were obscured by peculiarities in the natural cycle of the disease. In no case did the objective or subjective improvement following DFP equal or exceed that produced by neostigmine. Attempts to give larger doses of DFP resulted in marked nausea and vomiting, and symptoms referable to the central nervous system.

3. Despite the fact that DFP lowered plasma cholinesterase to zero, it produced less improvement than did neostigmine. The potentialities of a drug for treating myasthenia gravis clearly cannot be gauged by its effect upon plasma cholinesterase.

4. The possible reasons for the superiority of neostigmine over DFP in the treatment of myasthenia gravis are discussed.

5. As a practical treatment, DFP may be utilized in non-toxic doses to obtain its prolonged effect, at the same time that neostigmine is used to obtain its characteristically stronger action.

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CLINICAL AND ROENTGENOLOGIC ASPECTS OF COCCIDIOIDOMYCOSIS

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COCCIDIOIDOMYCOSIS is an infection caused by the fungus *Coccidioides immitis*. It was first reported in Argentina in 1892 by Posadas¹⁴ and Wernicke,²¹ and in this country by Rixford¹⁵ in 1894. Until 1936, the rather common and frequently fatal coccidioidal granuloma was the only phase of coccidioidal infection recognized clinically. At that time, and in subsequent publications, Gifford and Dickson^{8,9,11} identified "valley fever" or "desert fever" as the initial or primary infection with *Coccidioides immitis*. This had been a common but poorly understood endemic disease long familiar to residents of the San Joaquin Valley in California. Its relationship to coccidioidal granuloma was thus established and provided the basis for present knowledge of the pathogenesis and natural history of coccidioidomycosis.

So far as is definitely known, coccidioidomycosis in this country occurs in several rather sharply limited areas. These are characterized climatologically by long, hot, dry, dusty summers, and a low annual rainfall. They include the southern and western parts of the San Joaquin Valley in California, parts of southern Arizona and western Texas. Adjacent regions in Utah and New Mexico with similar climatic and geographic features have been regarded as probable endemic areas, but evidence establishing them as such is thus far inconclusive, and they have not been important sources of coccidioidal infection.

Until recently, coccidioidomycosis attracted little interest among physicians in this country for several reasons: (1) The source of the infection is confined to

a few sharply limited geographical regions. (2) Even in such regions, due to the mildness of the primary respiratory infection, many infected persons do not seek medical attention. Only the more severe or prolonged infections are likely to be seen by physicians, and in only a small percentage of these are the necessary facilities available for establishing the diagnosis. (3) Much that is new concerning coccidioidal infection from a clinical and roentgenologic standpoint dates from the fairly recent publications of Dickson and Gifford and has not yet diffused much beyond investigators and interested physicians, who encounter the disease in endemic areas.

As a result of war-time conditions, coccidioidomycosis has assumed considerable importance, and its endemicity is no longer an excuse for indifference regarding it. The Southwest has been an important army training area, and many installations have been established in localities where the disease is endemic. This situation has provided for a sustained increase in the incidence of the disease, due to the continuous influx of non-immune new arrivals into such areas. The likelihood of exposure is considerable and is increased with the duration of residence. The disease may be acquired in an endemic locality but manifested in a distant non-endemic region where it is unknown or unrecognized. Such a possibility confronts those in the military service, their families which in many instances accompany them, and innumerable civilians who have migrated to endemic regions where they are transiently employed in

various war-time projects. Not a few such areas have been and will continue to be attractive winter and health resorts with a large transient population of tourists and health seekers from various parts of the country. Thus, acute or chronic coccidioidomycosis may be encountered in individuals far removed from endemic areas. Moreover, inactive pulmonary residues, such as coccidioidal cavitation nodular pulmonary lesions may persist for months or years in apparently healthy persons who may or may not give a history of primary infection. The recognition and interpretation of such lesions is of the utmost importance if serious errors in diagnosis, treatment and prognosis are to be avoided. Without a doubt such lesions have been and will continue to be a basis for unwarranted compensation claims against the Government and might conceivably provide some problems in the realm of industrial compensation insurance. Knowledge of their significance and potentialities acquired by prolonged observation of such lesions is the only basis for an intelligent and equitable settlement of such claims. Army Medical Officers, Veterans Administration doctors and civilian physicians should be sufficiently familiar with the disease to recognize it in its various phases and especially its residual pulmonary lesions.

The purpose of this paper is to emphasize: (1) The increasing general importance of the disease and the necessity for its recognition wherever it may be encountered. (2) To review in the light of our own experience the salient clinical, laboratory and roentgenologic features of the disease. (3) To summarize the essential requirements of diagnosis and to indicate the relative value and limitation of various diagnostic procedures.

The present series consists of 77 cases admitted to an Army Air Force Regional Hospital in southern Arizona between August 1942 and August 1944, in which the diagnosis of coccidioidomycosis was confirmed either by coccidioidin serologic

tests or cultural studies. This number affords no basis for an accurate estimate of the incidence of coccidioidal infection at this installation. It obviously does not include asymptomatic infections or a considerable number of probable coccidioidal infections in which, however, the diagnosis was questionable. All cases were sporadic and were distributed throughout every month in the year. In the months from July through December, 57 cases (74.1%) occurred, the peak of incidence being in August (13%) and September (14.3%); 68.8% of the patients were white, and 31.2% were colored. The incidence among the Negro troops was 3 times that among the white in proportion to their relative number at this installation. The period of hospitalization averaged 42 days. 72 patients with acute, uncomplicated coccidioidomycosis recovered completely. Of 5 patients with dissemination, 2 recovered completely, and 1 with dissemination to the meninges has apparently recovered. Two patients with dissemination died. Ten (13%) had some type of residual pulmonary lesion revealed by Roentgen ray. Of these, 4 (5.2%) had pulmonary nodules, and 6 (7.8%) showed pulmonary cavitation. In addition to the 77 hospitalized patients studied, clinical and roentgenographic material was available on approximately 200 patients, chiefly out-patients with residual pulmonary lesions, observed in southern Arizona during the period from 1941 to 1945. These include military personnel, civilian dependents and civilian employees. When suitable, roentgenographic material from this group has been used to illustrate some of the late lesions of coccidioidomycosis.

Clinical Types of Coccidioidomycosis. Several clinical types of coccidioidomycosis are recognized: A. The initial or primary infection is always respiratory and incurred by inhalation of chlamydespores in contaminated dust. It is localized in the lungs, and in most instances subsides spontaneously and without sequelae. It may be entirely asymptomatic. This is

the usual type of infection among residents of endemic areas. Subclinical and unrecognized, its occurrence in an individual may be indicated only by demonstrable sensitivity to coccidioidin and uncommonly by residual pulmonary lesions revealed by Roentgen ray examination. Approximately 10 to 15% of individuals with 1 year's residence in an endemic area will acquire such infection, and after 10 years' residence approximately 80 to 90% will have acquired the infection.^{1,19} Of 200 enlisted men in the Medical Detachment of the hospital with up to 18 months' residence in the area and without a history of clinical infection, 12.5% reacted positively to the cutaneous coccidioidin test.

B. The type most frequently encountered clinically is an acute self-limited respiratory infection manifested predominantly as a bronchitis, pleuritis or pneumonitis. It is often clinically indistinguishable from more prevalent non-specific respiratory infections or primary atypical pneumonia, and diagnostic confirmation is not obtained ordinarily in civilian practice.

C. When the initial respiratory infection is associated with allergic phenomena such as erythema nodosum or erythema multiforme it may be easily recognized clinically and has been referred to variously as "San Joaquin Valley fever," "desert fever," "valley fever," and "desert rheumatism." Approximately 18% of the cases in our series were of this type—6.5% with erythema nodosum and 12% with other types of erythematous skin lesions. This incidence is slightly higher than that reported by Smith¹⁸ and is nearer the figures reported by Goldstein and Louie,¹² who found erythema nodosum in 19% of their patients and in approximately 25% of all cases there were cutaneous manifestations.

D. While complete recovery is the rule, in an unknown proportion of cases (13% in our series) the primary infection results in apparently benign, persistent residual pulmonary lesions, notably cavitation or nodules usually unaccompanied by constitutional symptoms or signs and

revealed by Roentgen ray examination of the chest.

E. In rare instances the initial infection does not subside but becomes progressive with widespread lymphatic and hematogenous dissemination. Any or all the organs of the body, with the notable exception of the digestive tract, may be involved. The lungs, tracheobronchial, supraclavicular and cervical lymph glands, the meninges, skin and bone are especially vulnerable. The clinical features and course may vary considerably, depending upon the extent of dissemination or the dominant localization. In the cases observed by us, the striking clinical manifestations were those of generalized miliary dissemination in 2, persistent massive pleural effusion in 1, pulmonary and glandular localization in 1, and meningeal in 1. Meningeal localization may be the only demonstrable site of spread or it may be simply a part of a generalized dissemination. The disseminated form usually runs a protracted course and terminates fatally in about one-half the cases. Miliary tubercles, confluent areas of caseation necrosis and typically granulomatous lesions occur in the involved organs. The disease may simulate and at times be indistinguishable, clinically and pathologically, from tuberculosis, differentiation, depending upon recovery of the etiologic agent.

It should be borne in mind that the disseminated phase, in the great majority of cases, develops sometime during the acute, primary infection and represents a continuous progression of the disease. Dissemination, if it is going to occur, usually takes place within the first few weeks or months, and thereafter its likelihood diminishes. It should be suspected in the presence of prolonged high fever or symptoms and signs indicative of meningeal, skeletal or cutaneous involvement. The coccidioidin serologic test is of help and may be decisive in detecting dissemination not clinically evident. Dissemination, after several years, as a result of endogenous reactivation, while admittedly a possibility, must

be extremely rare if it occurs at all. Present available evidence upon this point is meager and unconvincing.

Clinical Features. A. *Pain.* The most frequent, and in many cases, the predominant symptom in the acute infection is thoracic pain. It occurs in from 75 to 85 % of patients. In most instances (54.5 % in our series) pain is severe and pleuritis in character. In 2 instances patients were referred to the hospital from the dispensary with a diagnosis of fractured rib, although no history of injury had been elicited. When localized to the anterior chest, the pain may simulate anginal pain or the pain of acute pericarditis. In approximately 20 % of our patients the pain was substernal or constricting in character or described chiefly as a dull, heavy sense of discomfort. While the acute pain usually subsides after several days, it is not uncommon to note residual vague chest pains or aching, persistent or recurrent for periods of several weeks to several months.

Some patients with subpleural nodular lesions complained of a dull, boring ache, usually referred to the shoulder or interscapular region, in most instances unassociated with evidence of clinical activity. Such pain may persist with varying intensity for several months at a time with ultimately spontaneous subsidence.

B. *Fever.* Fever was present in the majority of patients sometime during the course of their illness. In the ordinary uncomplicated cases the average duration was approximately 1 week. However, the fever was occasionally prolonged, and in disseminated cases persisted for months. There was nothing characteristic about the fever curve. A fact to remember in this connection is that a considerable number of patients (39 % in our series) may be afebrile throughout the entire period of observation despite other symptoms and signs such as increased sedimentation rate and roentgenologic findings to indicate activity of the disease.

C. *Cough.* Less than half of our patients had any significant cough, and of these in only a small percentage was it produc-

tive. Sputum was usually scant, and even when obtainable examination for *Coccidioides immitis* was frequently unsatisfactory. Examination of fresh preparations is not reliable especially in inexperienced hands. Sputum examination is limited in usefulness by the infrequency with which satisfactory specimens are available in acute primary coccidioidomycosis and by the tedious cultural methods required to identify the organism. Failure to recover *Coccidioides immitis* from the sputum in cases with cavitation warrants an attempt to recover the fungus from gastric washing.

D. *Cutaneous Lesions.* The cutaneous manifestations associated with coccidioidal infection differ in character with the stage of the disease. In about 18 % of our cases with acute primary coccidioidomycosis, cutaneous eruptions occurred. These included the typical erythema nodosum of "desert fever;" located symmetrically over the pretibial region, and to a less extent the thighs, and referred to colloquially in the San Joaquin Valley as "the bumps." This type of eruption occurred in 5 (6.5 %) of the patients. In a larger proportion of cases, 9 (11.7 %), a less well-defined erythematous eruption occurred. Few of these were fairly typical erythema multiforme, but others were generalized, irregular, papular erythematous lesions involving the entire trunk and sometimes were distinctly morbilliform in appearance. The erythematous skin lesions usually occurred within the 1st week of the initial infection and subsided within approximately 1 to 2 weeks. Such lesions are associated with hypersensitivity to coccidioidin, development of a high degree of immunity and resistance to dissemination. In disseminated cases granulomatous skin lesions, verrucous or papillary in character, single or multiple cutaneous cold abscesses and indolent sinuses may occur. Two of our disseminated cases had such lesions. From them *Coccidioides immitis* may be recovered. They are invariably metastatic and indicate dissemination.

F. Arthritis. Arthritis has been reported as occurring in approximately one-third of the cases and accounts for the term "desert rheumatism." The involved joints have been described as tender, painful on motion, and at times slightly swollen. Effusion ordinarily does not occur. Goldstein and Louie¹² reported some dull articular ache or pain during the course of the illness in 50% of their patients. These observers noted no swelling or tenderness, and the roentgenograms were uniformly negative. Joint symptoms usually subside within a month and without residual damage or deformity. We have been singularly unimpressed with arthralgia as a significant complaint in any of our patients, and in none were objective joint changes encountered. In fact, arthralgia, beyond what might occur in a variety of acute infections, was not observed. Rosenberg, Dockerty and Meyerding¹⁷ state that joint symptoms are usually associated with erythema nodosum. However, Smith¹⁹ states that arthritis may occur without skin manifestations while the latter without arthritis are very frequent. Bone and joint lesions characterized by redness, swelling, fluctuation, adjacent skin lesions with ulceration or sinus formation are not infrequent in the disseminated form of coccidioidomycosis. However, we encountered none in our series.

While general symptoms of infection, such as headache, malaise, myalgia, lassitude and weakness occur, they are neither as frequent nor conspicuous as in most respiratory infections of comparable severity. This observation has been commented upon by others.⁵ The patients in general look and feel better than their clinical and roentgenologic findings would indicate. This is generally true, not only of the acute primary infections, but also of the disseminated form in which the disparity between the subjective complaints and objective findings has at times been striking. Hemoptysis is uncommon in the acute type of infection

although it occurs at times with residual cavitation.

G. Physical Findings. Physical findings in acute coccidioidomycosis in the absence of the cutaneous manifestations are of little or no help from a diagnostic standpoint. Despite pulmonary involvement, dyspnea, cyanosis, tachycardia and evidence of systemic toxicity are either absent or minimal. In contrast to the frequency of pleuritic pain, a pleural friction rub is seldom heard. Rarely, massive pleural effusion occurs, but in most instances the amount of fluid is insufficient to detect on physical examination. Signs of cavitation in the late cases are usually absent despite the size of some of the cavities. Hilar lymphadenopathy is a fairly common roentgenographic finding, but peripheral lymph gland involvement is not encountered in the acute cases. However, in disseminated cases, unusually extensive lymphadenopathy may occur. In such cases it is usually most marked in the hilar, supraclavicular and cervical groups and may progress to abscess formation with draining sinuses.

H. Laboratory Findings. Except for the cultural and inoculation tests to which we will refer later, laboratory studies have little in the way of specific value in the diagnosis of coccidioidomycosis. There are, however, a few findings that are helpful diagnostically or as an index of activity of the disease. The leukocyte count is usually moderately elevated. Eosinophilia has been noted and occurred in 18 (23.3%) of our patients. All of these had eosinophil counts higher than 10%, the highest eosinophilia being 29%. Moderate, to marked erythrocyte sedimentation rates were found in 75 of our patients (97.5%). This proved to be the most useful single index of activity and persisted in most instances long after complete subsidence of all the clinical findings with the exception of residual Roentgen ray findings.

There is some evidence to indicate that coccidioidomycosis occasionally may give rise to transient, false positive blood serologic tests for syphilis. Four patients in

our series had positive Kahn and Kline tests early in the course of their coccidioidal infection. In none was the Wassermann test positive. In 3 cases the duration of the positive serologic reaction persisted from 2 weeks to 1 month following which the tests became negative spontaneously. None of the patients had a history of syphilis, and clinical evidence was lacking in all cases. None of the more usual causes of false biologic positive tests could be demonstrated. In 1 case lues could not be excluded, although it was improbable. This patient (Case 3) had disseminated coccidioidomycosis, was critically ill, and although the Wassermann was negative, both the Kahn and Kline tests were 4+ on 3 different tests reported from different laboratories. Unfortunately verification tests were not obtained. Because of the possibility of syphilis complicating the coccidioidal infection, it was decided not to withhold antisyphilitic treatment. In view of subsequent experience, however, we feel that a longer period of observation would have revealed the true significance of the positive serologic tests in this case. The conclusion is obvious that great caution must be exercised in the interpretation of positive serologic tests for syphilis among patients with coccidioidomycosis. Reasonably prolonged observation and verification tests are indispensable in the solution of this problem.

Roentgenologic Features. GENERAL. Many physicians think of coccidioidomycosis as a disease which mimics tuberculosis. This generalization must be qualified by recognition of the fact that it is a disease which may simulate various types of acute pneumonic lesions in its earlier phases, and later may present film evidence which is similar to that in various forms of lymphoma, metastatic malignant lesions and primary malignant lesions. Bone lesions are absent in the primary phase of coccidioidomycosis, but in the disseminated type skeletal lesions may resemble various neoplastic lesions of the skeleton, non-tuberculous osteomyeli-

tis and periostitis and other destructive lesions of bone and cartilage in addition to tuberculosis.

Roentgenologic findings of this disease have been presented^{2-7,13,22,23} in medical literature of recent years. Comprehensive reviews of the literature are available in recent publications.^{5,7} It is desired to stress in this discussion certain salient features of roentgenographic nature and to summarize information obtained by the study of patients with acute and late coccidioidal pulmonary lesions. A tendency for primary pulmonary coccidioidomycosis to follow one of five general patterns has been observed on the basis of serial film studies extending over months or years. A finding of interest is the seasonal incidence of new cases as reflected by records of the Roentgenologic Service. For example, recently in 12 consecutive months, during the period from May to October inclusive, 75 new cases were observed, while during the months from November to April inclusive only 38 new cases were encountered. The short incubation period (8 to 15 days) accounts for the close correlation of Roentgen ray and clinical signs to the months during which hot dry weather is prevalent.

Roentgenologic Findings in Primary Coccidioidomycosis. In the primary phase of this disease it is well agreed that Roentgen ray findings are limited to the thorax. Patients may complain of arthralgia in the initial days or weeks of their illness, but film evidence of organic change in skeletal structures at this time is lacking. Roentgenologic findings are variable as summarized in the 77 hospitalized cases indicated in Table 1. Attention is called to a small group of patients (2.6%) who were hospitalized for coccidioidomycosis but in whom no Roentgen ray signs of the disease could be recognized despite knowledge of characteristic clinical findings such as erythema nodosum and evidence of activity of the disease by skin testing and by serologic studies. On the other hand, knowledge gleaned from single films or the aggregate of findings on serial

films sometimes identifies involvement as coccidioidomycosis before other positive evidence appears. From the Roentgen ray viewpoint an element of confusion arises from the fact that a patient may first be examined in almost any phase of the disease. If such a patient is filmed at the onset, the pulmonary findings may be exactly the same as would be expected from the acute bacterial and virus pneumonias. This group of early cases has presented a recurrent problem, which has been solved only by serial films and by the combination of roentgenographic and

oidomycosis the differential diagnosis on the basis of a single film may be impossible because of the similarity of the lesions to lymphoma, secondary malignant foci, or tuberculosis.

A. *Pneumonitis*. Acute pneumonitis in coccidioidomycosis may be nothing more than minimal increase in soft tissue prominence in one hilar area, or barely perceptible obscuration of hilar detail on one or both sides. This is a group of lesions which is easily missed on the basis of film evidence alone because of wide variations in prominence of hilar areas among normal



FIG. 1.—A, Acute bilateral pneumonitis. Minimal effusion, left. Hilar prominence bilaterally, predominantly left. B, After 15 days. Note tendency for nodular configuration opposite anterior end of 5th right rib. Persistent bilateral hilar accentuation. Increase in fluid accumulation in left pleural cavity. Findings disappeared after additional 3 months.

clinical findings to achieve accurate differentiation. One can safely state that early primary pulmonary coccidioidomycosis and primary atypical pneumonia are 2 conditions which may produce a variety of similar appearances in the lungs and pleure.

TABLE 1.—TYPES OF LESIONS IN PRIMARY COCCIDIOIDOMYCOSIS

	Per cent.
Pneumonitis	70 0
Adenitis	23 3
Cavitation	7 8
Nodules	5 2
Pleural effusion . . .	2 6
Negative chest film . .	2 6

If a patient is seen somewhat later in the primary phase of pulmonary coccidi-

persons. Aid is gained by comparison with the opposite hilus if the lesion appears to be unilateral, or by serial films, or both. More easily detectable pneumonic infiltration may be found in the perihilar area, or it may be subpleural. A dependable point regarding distribution of pneumonic foci is the fact that consolidation of an entire lobe is extremely rare. Multiple areas of infiltration may be visible in 1 lobe or in all lobes bilaterally at the time of onset of the disease (Figs. 1, 2, 3). Early pneumonic infiltration of lungs or hilar areas may resolve completely within 1 to 4 weeks. Commonly, complete resolution does not occur even though partial resolution is the rule

during the early weeks of the initial involvement. Once partial resolution has appeared the future pattern of involvement will depend on whether healing and fibrosis remain predominant or whether

about the time the initial acute pulmonary infiltration begins to recede (Figs. 1 and 2). A significant fact in connection with adenitis is that prominent nodes may appear in both the hilar and mediastinal



FIG. 2.—*A*, Admission film revealed scattered focal acute pneumonic infiltration in all lobes bilaterally, with slight bilateral hilar prominence. *B*, After 17 days. Pneumonic foci had partially resolved, with more nodular appearance. Hilar involvement, particularly on left, showed marked increase. Ten weeks later this patient showed no significant residue in the thorax.



FIG. 3.—*A*, Initial study of Nov. 14, 1942, revealed acute pneumonitis, lateral left mid-lung, right lateral subapical area, right base, and continuous with lower pole of right hilus. Mediastinal adenitis present. *B*, Film 8 months later shows residual cavitation opposite anterior end of 2nd right rib, residual nodule opposite anterior and 7th right rib, with decrease in hilar and mediastinal adenitis. Excavation, nodulation and adenitis were all observed in this 1 patient, with persistence of nodules and cavities for more than 2½ years.

focal necrosis of lung tissue occurs (see Chart 1).

B. Adenitis. Hilar and mediastinal adenitis are frequently observed with variable degrees of volumetric increase in soft tissue in these areas. Quite commonly the adenitis becomes more apparent at

areas, with subsequent complete return to normal and with no evidence of the disseminated phase at any time.

C. Cavitation. In observing patients with this disease one commonly encounters 2 distinct mechanisms of cavity formation (see Chart 1). A pneumonic

lesion may partially resolve, leaving peribronchial infiltration of fibro-exudative appearance, which seems to develop focal necrotic alteration. This type of cavity is ordinarily quite thin-walled and is likely irregular in contour (Figs. 4 and 5). These cavities frequently persist for more than a year and may vacillate in size to the extent of increasing in volume after having decreased markedly. Closure is assumed to occur as a result of predomi-

areas other than upper lobes in patients with this disease than in those with tuberculosis.

The other mechanism of cavity formation will be further discussed in connection with nodule formation.

An example of an irregular thin-walled cavity, which was regarded as similar to cavitation of coccidioidomycosis, is presented in Figure 6. Despite the similarity of film appearance, serial films and films

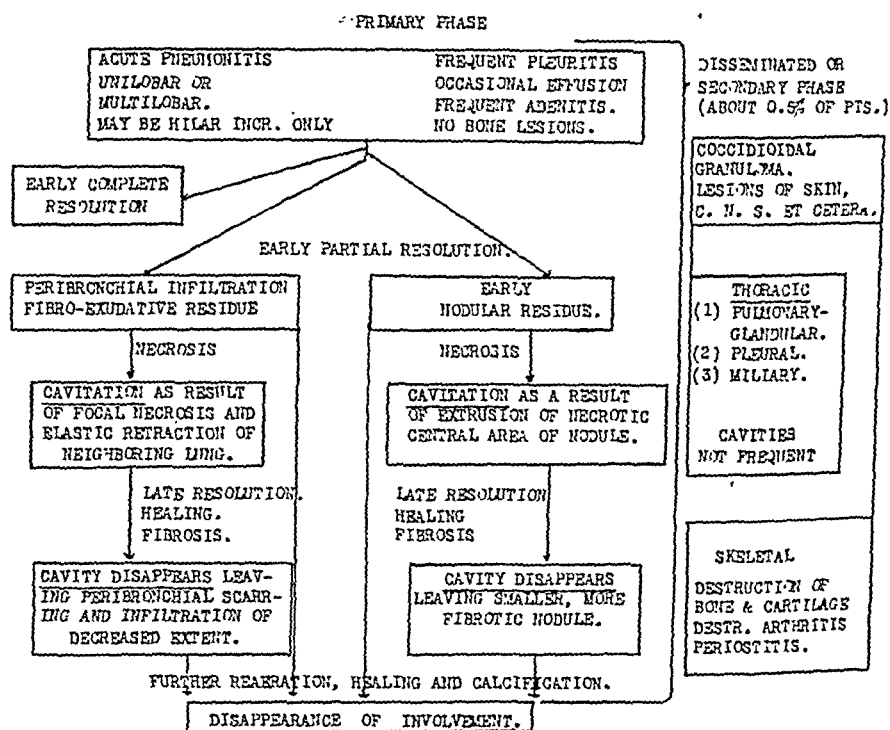


CHART 1.—Schematic representation of possible courses for resolution and progression of coccidioidomycosis as observed roentgenologically.

nance of fibrosis over further necrotic alteration and then bronchial occlusion with absorption of air from the interior of the cavity. Once complete closure occurs in a cavity of this type it is rare for it to reappear and ordinarily there is less tendency for appearance of a new area of excavation in the adjacent vicinity than would be true in tuberculosis. Another generalization in connection with cavity formation in coccidioidomycosis is the well-marked tendency for cavitation in

made prior to entry of this patient into an endemic area revealed that a pulmonary lesion existed before any contact with *Coccidioides immitis* was possible. The nature of the cavity was finally determined by repeated sputum studies and by the appearance of tubercle bacilli at a time when the patient experienced an intercurrent upper respiratory infection.

D. Nodules. If sufficient serial films are obtained on patients who have areas of pneumonic infiltration, one will fre-

quently observe nodule formation. These nodules correspond in location to a previously visible patch of pneumonic density of greater volumetric extent. Many of

sion of the center of such a nodule a cavity appears. A cavity of this type is commonly more nearly round in contour and is likely to have a thick wall (Figs. 7,



FIG. 4.—*A*, Excavation in area of previous fibro-exudative peribronchial residue. *B*, Same case 7 weeks later. Rapid disappearance of cavity with minimal residual peribronchial accentuation.

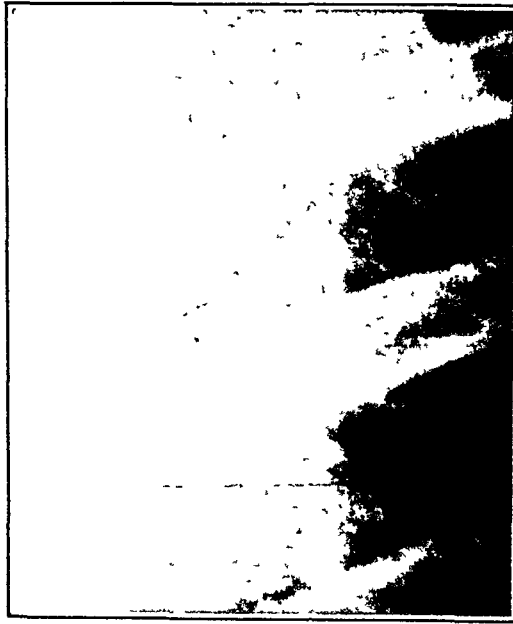


FIG. 5.—PA view of cavity in left upper lobe. Cavity known to have been patent for more than 18 months. Variable size of cavity at different times. This is the type of irregular thin-walled excavation which develops in areas of fibro-exudative peribronchial reaction rather than from necrosis of the center of a nodule.

these nodules slowly contract and over a period of months may disappear completely without trace. Other nodules evidently undergo necrosis and with extru-

8, 9). Cavities which appear in nodules do not ordinarily exceed 1 per patient. Again it is noted that cavities which arise in nodules are frequently encountered in

lower lobes and in the right middle lobe, rather than being limited to upper lobes. The appearance of cavitation in coccidioidomycotic nodules which otherwise close-

exclusion of metastatic carcinoma. Since these nodules have been observed to persist for more than $2\frac{1}{2}$ years, as in the example shown in Figure 3, it seems cer-



FIG. 6.—Cavity, right upper lobe, with minimal reaction. Onset of pulmonary symptoms before entry of patient to endemic area of coccidioidomycosis. Repeated sputum studies revealed acid-fast organisms.

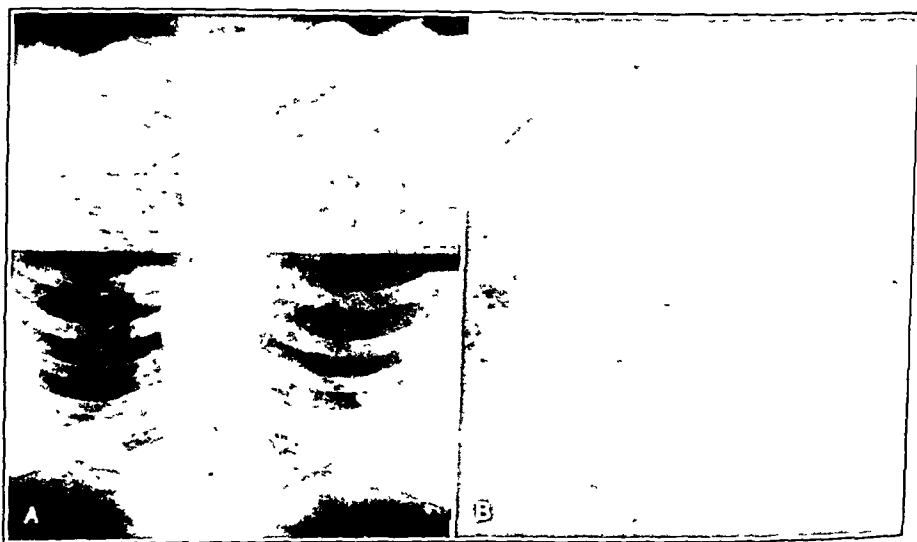


FIG. 7.—A, Large nodular lesion, with central excavation. "Doughnut" type cavity; split film, lordotic apical views in AP and PA positions. B, Enlarged view of cavity. Center of nodule no longer excavated 11 months later; size of nodule markedly reduced, indicative of predominance of healing and fibrosis over further necrosis.

ly resemble metastatic neoplastic lesions may be cited as a differential diagnostic feature (see Fig. 9). In non-excavating nodules there is often no film indication of etiology and no method for positive

tain that they will be observed in connection with planned mass surveys of chests to be accomplished in the coming years. A history of exposure to *Coccidioides immitis* and a positive coccidioidin

skin test in such patients may serve as a practical sorting routine in rounded or nodular soft tissue densities detected in lungs of patients who have been in

well-advanced fibrosis and good progress toward healing. Such deposition of calcium in a soft tissue nodular lesion of the chest would be an exceptional finding in



FIG. 8.—View of cavity in right upper lobe. Same patient as Figure 3. Patency of cavity for more than 2½ years observed in serial films.

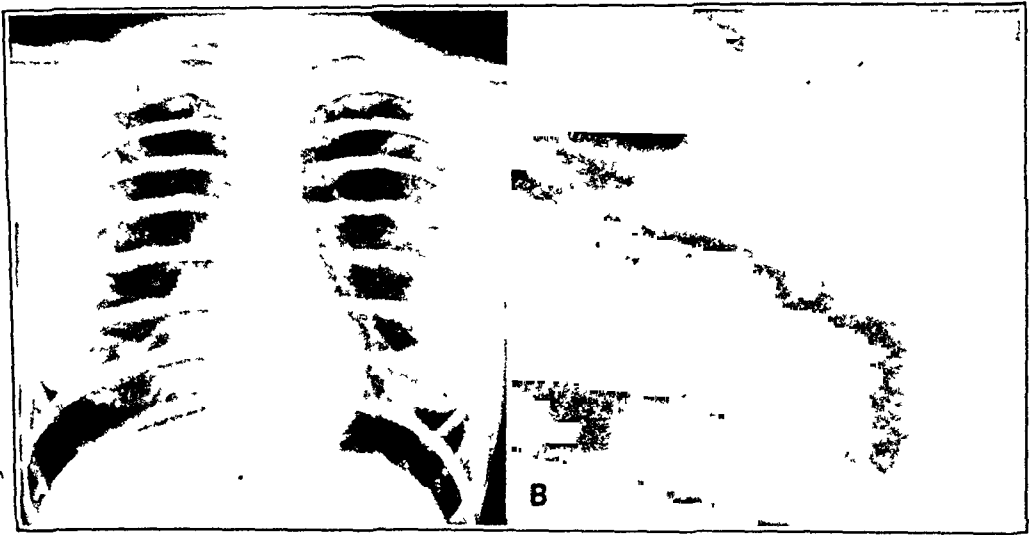


FIG. 9.—A, Nodule in lateral subapical region. Clinical signs of active coccidioidomycosis. B, Detail view to show excavation in nodule. One year later this patient, a female age 24, was found to have a scirrhous carcinoma of the stomach. Excavation in center of pulmonary nodule and positive clinical findings of coccidioidomycosis aid in excluding the possibility of a metastatic neoplastic lesion.

endemic areas, and thus avoid unnecessary hospitalization and diagnostic procedures.

One frequently observes a characteristic central calcific stippling in soft tissue nodules of coccidioidomycotic origin (Fig. 10). This phase of nodulation indicates

malignant metastatic foci. A similar phenomenon can occur in osteogenic sarcoma metastatic to the lungs, but calcium formed in such metastases is likely to be evenly distributed character throughout the metastatic nodule, while in coccidioidomycosis the calcium is more stippled

in appearance and more likely confined to the center of the nodule.

Cavity closure following necrosis of the center of a nodule appears to come about by a predominance of healing and fibrosis over further necrosis and concentric constriction of the cavity to form a nodule

or weeks of the illness. As indicated in Table 1, effusions are quite uncommon. This statement refers to effusion detectable on a routine chest film. Rigos,¹⁵ in reporting a roentgenographic study of pleural effusion, called attention to the fact that an effusion of approximately



FIG. 10.—Characteristic pattern of central calcific deposition, as observed in some of the pulmonary nodules of coccidioidomycosis as healing progresses. Note non-calcified soft tissue periphery.

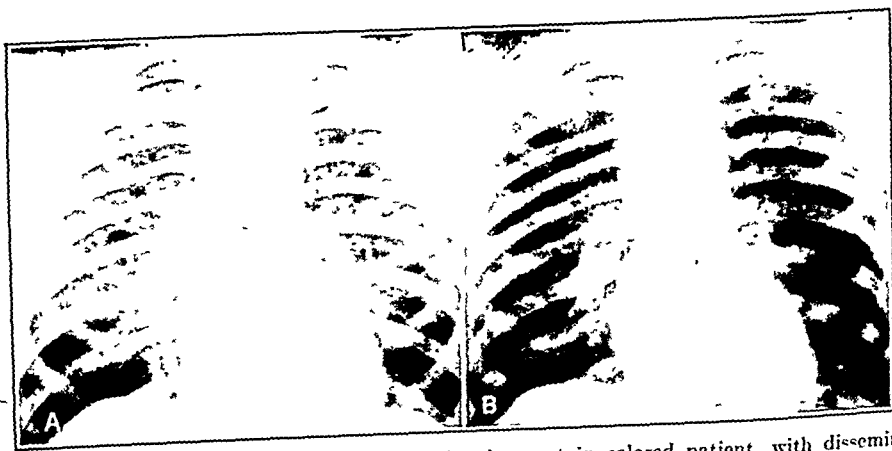


FIG. 11.—A, Hilar and mediastinal glandular involvement in colored patient, with disseminated coccidioidomycosis and extensive peripheral adenitis. Granulomatous lesions were present in the skin. B, Persistent mediastinal adenitis 6½ months later. Increased density, left supraclavicular area, from peripheral lymph node enlargement. No evidence of pulmonary cavitation.

of smaller size than the original one, or bronchial occlusion with absorption of air content, or both.

E. Pleuritis. Diffuse pleural thickening, associated with acute pneumonic lesions of coccidioidomycosis is a common finding. The pleural reaction of the primary phase ordinarily subsides during the early days

400 cc. or less may be present without producing significant alteration in film appearance on a standard 14 x 17 inch chest film made with the patient erect. so that small degrees of effusion may well occur more frequently than indicated in Table 1. Pleural reaction with effusion is represented in Figure 1.

Progressive or Disseminated Coccidioidomycosis. About 0.5% of patients who acquire clinical primary coccidioidomycosis eventually develop the progressive or disseminating type of infection. This percentage level is directly proportional to the number of racially predisposed individuals in the group considered. Obviously if a large percentage of exposed population is of the Negro race, the incidence of disseminated coccidioidomycosis will be much greater. Nodules, cavities and adenitis of the late primary phase are not to be confused with the disseminated phase of this disease.

A. *Lungs.* In the disseminated phase thoracic lesions may be predominantly hilar and mediastinal adenitis, as shown in Figure 11. Disseminated involvement in this patient (Case 2) was manifested by peripheral adenitis and granulomatous skin lesions and draining sinuses. A miliary distribution of lesions may occur, closely simulating miliary tuberculosis of the lungs. In disseminated cases, pleural reaction may be a predominant feature (Case 3).

B. *Bones.* Coccidioidomycotic skeletal involvement is likely to appear in multiple bones with the probability of associated multiple joint lesions. Lesions are primarily destructive, involving bone and cartilage and producing an appearance similar to tuberculosis. Benninghoven and Miller,² in reviewing 500 reported instances of bone and joint lesions due to *Coccidioides immitis*, found the greatest involvement in the spine, pelvis, hands and lower extremities.

C. *Central Nervous System.* Meningitis as a result of coccidioidomycosis is a common occurrence if dissemination does occur. Martin¹² has suggested that meningeal and spinal cord lesions may be more effectively evaluated by ventriculography and myelography respectively.

Roentgenologic features to be emphasized as a result of the present study include the following:

1. From roentgenographic point of view the differential diagnosis of coccidioido-

mycosis is not limited to tuberculosis. Early lesions may simulate many acute pneumonic processes. Late lesions may simulate various forms of neoplastic disease.

2. Serial film studies of patients with coccidioidomycosis frequently aid in identification of this condition when one bears in mind the 5 common patterns of progression and resolution presented here schematically.

3. Hilar and mediastinal adenitis of considerable extent may occur in the primary phase of coccidioidomycosis, with gradual spontaneous disappearance and without entry of the patient into the disseminated phase.

4. Two distinct mechanisms of cavity formation are prevalent in coccidioidomycosis during the primary phase. Cavitation is relatively less frequent during the disseminated phase.

5. Coccidioidomycotic cavities and nodules not uncommonly persist for many months or several years and must be included in the differential diagnosis of such lesions to be encountered in the planned mass surveys of the chest.

Diagnosis of Coccidioidomycosis. In endemic regions the possibility of coccidioidomycosis is always present, and there has been a tendency to make the diagnosis on insufficient grounds. Too frequently a patient with or without a respiratory infection but a positive coccidioidin cutaneous test has been so diagnosed without cultural or serologic confirmation. A diagnosis on such evidence is always questionable. On the other hand, acute primary coccidioidomycosis acquired in an endemic area but manifested elsewhere will, in all probability, be incorrectly diagnosed. If the late residual pulmonary lesions are unrecognized or misinterpreted, the likelihood of serious error in diagnosis, prognosis and treatment is great. Considering the differential diagnostic possibilities presented by pulmonary nodules or cavitation, especially metastatic malignancy and pulmonary tuberculosis, such errors may be personally, professionally

and economically disastrous. The disseminated form may be confusing if one is unfamiliar with its protean manifestations.

Absolute proof of coccidioidal infection depends upon tissue, cultural and animal inoculation studies. It requires: (1) observation of the characteristic doubly contoured, non-budding endosporeulating spherules in tissue sections, sputum, pus, pleural or spinal fluid; (2) recovery of the mycelial form from culture on Sabouraud's or differential media; and (3) demonstration of the spherules in inoculated animals. For the latter purpose, injection into a mouse or guinea pig is made, usually intraperitoneally. Direct smears are not reliable. Gastric washings or bronchoscopy may afford material for examination. When gastric washings are used, examination must be done promptly as the spherules are digested by the gastric secretion.

For practical purposes, positive identification of the fungus obtained by the above procedures is rarely necessary and may be difficult or even impossible in acute primary coccidioidomycosis, in which more often than not the fungus cannot be demonstrated. In such cases, sputum may be entirely lacking, and there are no accessible lesions to afford a source of recovery of the fungus. A diagnosis in the acute phase can be made with reasonable certainty and minimal error if the following criteria are fulfilled: (1) recent residence in an endemic region; (2) an acute respiratory infection with or without erythematous cutaneous lesions; (3) a positive coccidioidin cutaneous test; and (4) a positive coccidioidin serologic test. Additional evidence may be afforded by roentgenologic findings. An initially negative cutaneous test with a subsequent positive test is conclusive evidence of coccidioidal infection. The additional finding of eosinophilia increases the probability of coccidioidomycosis.

Cutaneous Coccidioidin Test. This test has been of great value in the diagnosis of coccidioidal infection. Material for the test was provided by Dr. C. E. Smith

of the Department of Public Health and Preventive Medicine, Stanford University. The technique and precautions have been fully described by a number of writers.^{5,7,19} A dilution of 1 to 1000 was originally used by us for routine testing. However, it became apparent that the use of this dilution resulted in missing some infections positive in 1 to 100 dilution. Thereafter, the latter was used routinely except in patients exhibiting erythema nodosum. Such patients are hypersensitive to coccidioidin, and in some instances respond with violent local and even systemic reactions. Occasionally 1 to 10 dilution is indicated if disseminated coccidioidal infection is suspected. There is no evidence that reactivation of old focalized lesions or dissemination can occur as a result of the coccidioidin cutaneous test.⁷ The test may be negative in early primary coccidioidal infection, but upon repetition will almost invariably become positive in undisseminated cases. It is important to consider the time element between the onset of the disease and the time at which the coccidioidin cutaneous test is performed. If done too early and not repeated, one may acquire the false impression that coccidioidal infection has been excluded. On the other hand, an initial negative test followed by a subsequent positive test is practically conclusive evidence of coccidioidal infection. In 11 of our cases the test was negative on admission and did not become positive until from 2 to 3 weeks later. The test may be negative in disseminated coccidioidomycosis. This was true in 3 of our disseminated cases. In these it remained negative even in 1 to 10 dilution throughout the entire period of observation despite clinical, serologic and cultural evidence of the disease. This is a fact of considerable diagnostic importance as a negative test may be indicative of anergy and not of the absence of coccidioidal infection. A positive test has the same diagnostic significance and limitations as the tuberculin test. It is not acceptable evidence of active coccidioidal infection.

Usually sensitivity to coccidioidin exists indefinitely. However, it is said to diminish or even gradually disappear in some individuals.⁷ We have no evidence upon this point. There is some evidence that cross-sensitivity to coccidioidin may exist. Emmons¹⁰ found this to be the case with *Haplospoangium parvum*, a fungus prevalent in parts of Arizona. Smith¹⁹ states that some individuals from Missouri, Illinois, Michigan, Indiana, Tennessee, Kentucky, Ohio, the Virginias, Pennsylvania and New York in which coccidioidomycosis is not known to be endemic show some borderline sensitivity to coccidioidin. However, from a practical clinical standpoint, the test is specific and quite reliable. A positive cutaneous reaction indicates permanent immunity to exogenous reinfection. The skin test is of importance in the presence of pulmonary cavities with negative serologic evidence. In such instances, however, only the recovery of the organisms is decisive. The diagnosis of coccidioidal cavitation can only be presumptive in the presence of a positive coccidioidin cutaneous test but with negative cultural findings.

Coccidioidin Serologic Test. Precipitin and complement fixation tests, as performed by Dr. C. E. Smith, using coccidioidin as antigen, are of value in the diagnosis and prognosis of coccidioidal infections. Obtaining antigen which is consistently satisfactory for complement fixation has delayed publication of these procedures.¹⁹ In mild infections and in the late quiescent or inactive phases, these tests are usually negative. In the active phase of coccidioidal infection they are positive, and the more severe the infection, the more likely it is that precipitins and complement fixing antibodies will develop. In the ordinary, uncomplicated infections precipitins are present in higher titer than complement fixing antibodies and tend to disappear earlier. The titer of complement fixing antibodies parallels the severity of the infection, and in certain ranges is indicative of impending or actual dissemination. Thus, the

serologic test provides a reliable index of the severity of the infection, and to this extent has prognostic as well as diagnostic value. A diagnosis of dissemination should not be made in the absence of specific antibodies in relatively high titer.⁷ In late, inactive phases of coccidioidal infection, characterized by residual pulmonary cavitation or nodular focalization, the serologic tests have been uniformly negative in our experience even when the fungus could be recovered from the sputum. Serologic tests should be performed whenever dissemination is suspected, regardless of the results of the cutaneous coccidioidin test.

Five patients in our series had clinical, serologic or cultural evidence of dissemination. Two (a 35 year old white private, and a 30 year old colored private) died in from 4 to 5 months. Necropsy, in both instances, revealed miliary dissemination in the lungs, tracheobronchial lymph glands, liver, spleen and kidneys and indolent cutaneous abscesses and verrucous skin granulomata. One case in addition had involvement of the meninges, left ulna, tibia, and knee joint. The clinical and gross pathologic findings in these patients were practically indistinguishable from miliary tuberculosis.

Three disseminated cases recovered. The dominant clinical pattern (namely, meningeal, pulmonary-glandular and pleural) was different in each and likewise differed from the generalized, miliary type which characterized the two fatal cases. For this reason it is felt that a brief clinical summary of these cases will serve to emphasize: (1) the protean features of disseminated coccidioidomycosis, (2) the possibility of recovery in even the most critically ill; (3) and the utter fallacy of evaluating therapeutic measures in individual cases.

Case Report. CASE 1. A 31 year old Negro private entered the hospital Feb. 9, 1943, because of a non-productive cough of 2 weeks duration. Physical examination was negative except for crepitant râles at the right lung base. He had lost 16 pounds in the

2 months prior to admission. A roentgenogram of the chest revealed widening of the right hilar region and irregular mottled density in the right lower lung field, interpreted as probable atypical pneumonia. The day following admission, he developed fever, ranging from 101° to 103° and continuing for 1 month. On Feb. 11, 2 days after admission, the coccidioidin cutaneous test was + in 1 to 100 dilution. On February 14, the leukocyte count was 10,300 with 10% eosinophils. The Kahn test was negative. On February 21, blood obtained from the patient for coccidioidin serologic test was sent to Dr. C. E. Smith at Stanford University School of Medicine. The test was reported as follows:

Complement fixation: serial dilutions of serum (0.25 cc.): 1 to 2, 4+; 1 to 4, 4+; 1 to 8, 4+; 1 to 16, +; 1 to 32, +.

Precipitin tests: serial dilutions of antigens: undiluted, 4+; 1 to 10, 4+; 1 to 40, +; 1 to 100, 0.

These findings were characteristic of severe coccidioidal infection. On March 12, the density in the right lung field was not appreciably changed but there was marked enlargement of the mediastinal shadow indicative of right hilar adenopathy. At this time the fever subsided and the patient was generally improved. On March 20, the second coccidioidin serologic test, performed at Stanford, was reported as follows:

Complement fixation: serial dilutions of serum (0.25 cc.): 1 to 2, 4+; 1 to 4, 4+; 1 to 8, 4+; 1 to 16, 4+; 1 to 32, 4+; 1 to 64, 3+; 1 to 12, +; 1 to 156, 0.

Precipitin tests: serial dilutions of antigens: undiluted, 3+; 1 to 10, 3+; 1 to 40, 0; 1 to 100, 0.

The increase in the complement fixation titer was interpreted as indicating dissemination despite the normal temperature and absence of clinical evidence of dissemination. About April 20, 10 weeks after admission to the hospital, the patient suddenly developed severe frontal and occipital headache with nuchal rigidity. The cerebrospinal fluid pressure was moderately elevated (manometric readings were not obtained). The fluid contained 900 cells per c.mm., of which 70% were lymphocytes. Cerebrospinal fluid, Kahn and Wassermann were negative. No organisms were found on smear or culture. Following the onset of meningeal symptoms the patient ran a

low-grade fever for 5 days. This subsided without recurrence. About Apr. 25, 1945, the spinal fluid showed 530 cells, of which 83% were lymphocytes and 17% neutrophils. The colloidal gold curve was 555551-0000. The patient was afebrile at the time but had severe generalized headache, and the nuchal rigidity persisted. There were no localizing neurologic findings at any time during the period of observation. The ocular fundi were consistently normal and at no time were signs of cerebrospinal fluid block demonstrable. On May 26, 1943, approximately 1 month after the occurrence of his meningeal symptoms he was transferred to William Beaumont General Hospital. At that time headaches were his only complaints. These were described as diffuse and of moderate intensity. They were not incapacitating, and the patient could not be convinced of the necessity for remaining in the hospital. His general condition was good, and he had gained considerably in strength but was still 20 pounds below his average weight. Repeated detailed neurologic examinations did not reveal abnormal findings other than a slight to moderate nuchal rigidity. On June 9, cerebrospinal fluid was examined again at William Beaumont General Hospital, and the following findings were reported: white blood cells, 586 (lymphocytes, 58%; neutrophils, 42%). Globulin was 3+, gold curve 5555543210. At this time the fluid was reported positive for *Coccidioides immitis*, both on direct smear and culture. Guinea pig inoculation, unfortunately, was not done. About June 20, approximately 2 months after dissemination to the meninges, the patient first noticed a slight staggering on walking and transitory diplopia which continued off and on for approximately 1 month. On July 5, the cerebrospinal fluid was again reported positive for *Coccidioides immitis*, both on direct smear and culture. The total cell count was 725 (95% of which were lymphocytes). The colloidal gold curve was 5555555421. At this time the patient complained only of headaches and the inactivity that was forced upon him. He had a rather marked feeling of euphoria and appeared normal. Detailed physical examination revealed no signs of dissemination to other organs. Neurologic examination was repeatedly negative. On September 17, he was transferred to a Veterans Administration Facility. There he was

reported as well-nourished, ambulatory and asymptomatic, and apparently normal in all respects. Physical and neuropsychiatric examinations were again essentially negative. Laboratory findings, including blood count, blood Wassermann, sedimentation rate and sputum examination were normal. Chest Roentgen ray, September 1, showed fibrosis involving the right lower lobe and enlargement of the right hilar glands. The cerebrospinal examination, October 1, was reported negative at this institution. On October 5, the patient was given a pass and failed to return to the hospital. He was discharged "Absent Without Leave." Periodic follow-up of this patient has been obtained. The last report (Aug. 15, 1945) reveals that the patient has remained well, is without complaints and is currently employed as a stevedore in Norfolk, Virginia.

Comment. It may be objected that absolute proof that this patient had coccidioid meningitis is lacking in that the etiologic agent was not identified by animal inoculation studies. However, the clinical and laboratory evidence supports the diagnosis beyond a reasonable doubt. The diagnosis of disseminated coccidioidomycosis is conclusive, being based upon the following evidence: The patient, a colored man, living in a known endemic region, developed a respiratory infection, characterized by cough, fever, roentgenologic signs of pulmonary infiltration and hilar adenopathy, eosinophilia, coccidioidin skin test positive in 1 to 100 dilution and 2 coccidioidin serologic tests with complement fixing antibodies present in high titer indicative of dissemination. During the course of disseminated coccidioidomycosis the patient developed signs and symptoms of meningitis confirmed by repeated cerebrospinal fluid lymphocytosis, a parietic type of gold curve on 3 examinations (strong corroborative evidence in the absence of syphilis) and organisms identified by direct smear and culture as *Coccidioides immitis*.

The patient's course has been entirely benign, and he remains asymptomatic and in apparently good health. In view of the fact that no authenticated recovery from coccidioid meningitis has been reported and that survival for long periods occurs and complications incident to cerebrospinal fluid block may be long delayed, the case is presented not as one of recovery, but one with a survival period of 2 years and 7 months and who has long been free from symptoms of any kind.* The patient will be followed from time to time and a report of his future course will be made in a subsequent communication.

CASE 2. A 22 year old Negro private, first class, was admitted to the hospital on Dec. 14, 1943, because of progressive cough and expectoration, profound lassitude, weakness for 2 months, and pain in the chest of 2 days' duration. Physical examination was essentially negative except for fever, diffuse sibilant râles bilaterally, and a small indolent verrucous skin lesion in the anterior midline of the neck. The chest roentgenogram on admission showed marked tracheobronchial lymphadenopathy and extensive bilateral pulmonary infiltration. The erythrocyte count was normal, but there were 15,500 leukocytes with a normal differential count. On December 15, the Kahn test was 3+. On December 19 it was 1+ and on December 31 it was reported as doubtful. Subsequently, reports on the Kahn and Kline tests from 3 different laboratories varied from negative to 1+. After Jan. 7, 1944, the Kline, Kahn and Wassermann tests were repeatedly negative. Spinal fluid examination was negative. The coccidioidin cutaneous test in 1 to 100 and 1 to 10 dilutions were negative repeatedly over a period of 5 months. *Coccidioides immitis* was not recovered from the sputum, but biopsy and culture from the skin lesion on the neck were positive for the fungus. Serologic tests done at Stanford confirmed the diagnosis of disseminated coccidioidomycosis. The patient ran a spiking fever from 101° to 103°

* Dr. C. E. Smith, in a personal communication, cites an instance of proven coccidioid meningitis with survival for 4 years. During this period, however, the patient continued to have symptoms and ultimately died as a result of internal hydrocephalus, a late sequel of his meningitis. Although the infection apparently was inactive mechanical cerebrospinal fluid block presumably was responsible for the patient's death.

daily until May, and thereafter between 99° and 100° for approximately another 2 months.

In January, he developed a second papillomatous skin lesion about the right nares. This enlarged to approximately the size of a walnut, was dry and irregular, without acute inflammatory reaction, and showed numerous white, punctate areas on the surface. *Coccidioides immitis* was recovered from the lesion. In February, the lymph glands at the posterior angle of the jaw on the right side became enlarged. Subsequently, there was extraordinary swelling, tenderness and fluctuation in the right pre-auricular, submental and bilaterally in the cervical and supraclavicular lymph glands. The latter progressed to abscess formation. These were incised, and for 2 weeks continued to drain a thin, turbid fluid with curd-like material from which *Coccidioides immitis* was cultured. In May, there was a reduction in the fever and gradual regression of the lymphadenopathy, gain in weight and progressive clinical improvement which continued until his transfer to another hospital on July 2. He was discharged from the latter and from the Army on August 3 greatly improved. His clinical improvement could not be correlated with attempts at specific therapy including 3 immunotransfusions and a course of penicillin. It is possible that the latter might have prevented secondary infection of the draining abscesses as these were conspicuously free from pyogenic infection and healed uneventfully.

Comment. The dominant clinical features in this case were the high, irregular fever with gradual subsidence over a period of 7 months, extensive bilateral pulmonary infiltration, extraordinary hilar and cervical and supraclavicular lymphadenitis with abscess formation and drainage in the latter, verrucous skin lesions and a transient positive Kahn test. A positive coccidioidin cutaneous test (even in 1 to 10 dilution) was never obtained on this patient, although the coccidioidin serologic test was positive in high titer, characteristic of dissemination. Although the patient was critically ill and originally given a poor prognosis, his symptoms and findings finally regressed and spontaneous recovery occurred.

CASE 3.—A 31 year old white private entered the hospital on Nov. 20, 1942. He had been in Arizona for 2 months. He gave no history of serious illness, venereal or otherwise. One week prior to admission he developed cough, fever and pleuritic pain in the left side of the chest. Physical examination revealed only crepitant râles at both lung bases. The temperature returned to normal on the 5th day and remained so until December 1. At that time, in addition to cough and chest pain, fever of a high swinging type ranging from 100° to 104° recurred, associated with tachycardia, drenching sweats and clinical and roentgenologic signs of massive left-sided pleural effusion. There was an extreme shift of the mediastinum to the right associated with episodes of distressing dyspnea and cyanosis, relieved by repeated thoracentesis. A leukocytosis between 21,000 and 30,000 was consistently present for 2½ months. Eosinophilia ranged between 10 and 27%. The pleural fluid was serous and varied in specific gravity from 1.019 to 1.024, had a high protein content and contained 500 to 600 cells per c.mm., predominantly lymphocytes. No organisms were recovered from the fluid. Serologic tests for syphilis were of interest. They were reported as follows: November 21, Hahn 4+, Kline 4+; November 26, Kahn 4+, Kline 4+; December 3, Kahn 4+, Kline 4+, Wassermann negative. The cerebrospinal fluid was negative. The patient's condition was critical, and because of the possibility of complicating syphilitic infection, antiluetic treatment was begun and continued for 26 weeks. In the light of subsequent experience it is felt that these serologic tests were of the false biologic type and not indicative of actual syphilitic infection. The coccidioidin cutaneous tests in dilution of 1 to 100, or above, were repeatedly negative. The test in 1 to 10 dilution was slightly positive (+). On Apr. 8, 1943, the coccidioidin serologic test was positive in high titer, indicative of dissemination. Numerous sputum examinations were negative for tubercle bacilli and fungi.

The patient was transferred to Fitzsimons General Hospital on February 12. On April 12, *Coccidioides immitis* was recovered from gastric washings and identified by positive guinea pig inoculation. The patient remained critically ill from Dec. 1, 1942, until early in March 1943, following which

there was a gradual regression of all his symptoms and general clinical improvement. At no time did he have clinical evidence of syphilis. He was given a Medical Disability Discharge on June 21. Reports of examination at a Veterans Administration Facility, Dec. 3, 1943, a year after the onset of his illness, indicated complete recovery. The blood Wassermann at that time was negative. No evidence of pulmonary residue or pleuritis was found clinically or roentgenologically.

Comment. The salient features in this case include fever, cough, pleuritic pain, massive pleural effusion with compression symptoms, high sustained leukocytosis, eosinophilia, positive blood Kahn and Kline tests but negative Wassermann; only slightly positive cutaneous coccidioidin tests in 1 to 10 dilution; coccidioidin serologic tests characteristic of dissemination, and recovery of *Coccidioides immitis* from gastric washings. The patient's illness simulated tuberculous pleurisy with effusion, but presented certain hematologic and serologic features not explicable on such a basis. The etiologic diagnosis was established by the coccidioidin serologic tests and confirmed by animal inoculation studies. No localization of the infection other than pleural could be demonstrated. Unfortunately, premature antiluetic therapy instituted because of the urgency of the situation masked the significance of the positive Kahn and Kline tests. However, subsequent experience with false positive serologic tests in coccidioidomycosis leads us to believe that syphilis was improbable in this patient, and that antiluetic treatment should have been withheld until the problem of co-existent syphilis could be decided by further observation and verification tests.

Differential Diagnosis. The differential diagnosis of coccidioidomycosis would require consideration of a variety of unrelated and dissimilar diseases. Many of these have only a superficial resemblance to certain features of coccidioidomycosis. From a practical, clinical standpoint acute coccidioidomycosis may simulate bronchi-

tis, pleuritis, influenza and the bacterial pneumonias. In our experience the most common differentiation was from primary atypical pneumonia. If, as occasionally happens, the initial cutaneous eruption is prominent, it may dominate the clinical picture for a time and suggest measles, scarlet fever, drug idiosyncrasy or diseases of the erythema group. The association of fever, arthritic pain, erythema nodosum, increased erythrocyte sedimentation rate, pleural involvement and pneumonitis might conceivably be mistaken for rheumatic fever. When eosinophilia is marked, parasitic infestation, trichiniasis and even Loeffler's syndrome might be suspected. Practically, these should rarely if ever cause difficulty.

The disseminated form is more likely to be confusing because of the number and variety of manifestations possible in the respiratory, nervous and skeletal systems and in the skin and lymph glands. It must be differentiated from other mycotic infections and from miliary, pulmonary, meningeal and bone tuberculosis. Localized intracranial granuloma simulating brain tumor has been reported.^{18,20} In many instances the residual, pulmonary nodular lesions observed roentgenographically are not easily distinguishable from malignant metastases. A knowledge of the multiplicity of clinical features, of the value and limitations of the cutaneous and serologic tests, and cultural and animal inoculation studies should enable one to establish the diagnosis in the overwhelming majority of cases.

Treatment of Coccidioidomycosis. The object of treatment is to afford the patient the optimum conditions for focalizing his infection. This requires adequate rest, general measures and symptomatic treatment such as are carried out for any patient with an active infection. Such measures should be maintained until the infection has subsided clinically and the pulmonary lesions have regressed completely or become stationary. Prolonged hospitalization may be required. The sedimentation rate affords a reliable index

of activity of the infection. Special caution should be exercised in evaluating activity in dark-skinned patients such as Negroes, Filipinos and Mexicans, who have a greater susceptibility to infection and a higher incidence of dissemination. Drug treatment is of little importance. Symptomatic use of analgesics such as salicylates, with or without codeine, suffice in the ordinary acute cases.

There is no specific treatment for disseminated coccidioidomycosis. The variety of drugs used in the past, including the more recently tried sulfonamides and penicillin, have proven ineffective. Although some have advocated the use of vaccines, their value is still unestablished. Immuno-transfusions have been carried out, but their therapeutic efficacy is difficult to assess and their specific value is doubtful. Incision and drainage of abscesses with concurrent use of penicillin to minimize secondary infection can be undertaken with impunity. These lesions appear to heal satisfactorily under such circumstances. Surgical removal of fungating or verrucous cutaneous lesions has been done with good results. It is obvious, however, that the removal of such lesions exerts no influence on the fundamental course of the disease.

Treatment of patients with pulmonary cavities is still unsettled. Such patients are apparently immune to dissemination and evidence of local spread of the fungus from cavities to adjacent pulmonary tissue has not been demonstrated. Most cavities ultimately heal spontaneously. There is a difference of opinion as to whether or not active treatment should be used even when the cavities persist for long periods. Patients with such lesions are usually in good health and suffer no apparent ill-effects, except occasional hemoptysis in a small percentage of cases. Conservative measures seem to be the treatment of choice. We have had no experience in the use of pneumothorax, phrenicocolysis or the application of weighted bags to the chest as suggested by Denenholz and Cheney.⁷ We have en-

countered no patients in whom it was felt that any of these measures were indicated.

Summary. As with many diseases which hitherto have been of merely local or academic interest, coccidioidomycosis, due to the exigencies of war, has acquired a more general and practical clinical importance. A large influx of soldiers and civilians into endemic regions had occurred. Several such areas have been and will continue to be popular health and winter resorts, attracting patients and tourists from various sections of the country. A number of non-immune new arrivals dependent upon their length of residence, inevitably acquire the infection and subsequently move elsewhere. Many such individuals, with or without a history of primary infection show residual pulmonary lesions roentgenographically. The interpretation of such lesions presents considerable difficulty and possibilities for serious error if their nature and significance are unknown. The necessity and responsibility for recognizing coccidioidomycosis in its various phases and wherever encountered is evident.

The present report is based upon personal experience with 77 hospitalized cases of coccidioidomycosis and a study of clinical and roentgenographic material upon approximately 200 additional patients observed in southern Arizona between August 1941 and August 1945. The salient clinical, laboratory and roentgenographic features are presented with particular emphasis upon changes and variations peculiar to different types and stages of the disease. The common Roentgen ray patterns of progression and resolution are described. The fact that coccidioidomycotic cavities and nodules may persist for many months or several years is reiterated, and in the future must be taken into consideration in contemplated mass surveys of the chest. The essential criteria for diagnosis, and the relative value and limitations of various diagnostic procedures are indicated. Three case histories, illustrating different clinical pat-

terms of disseminated coccidioidomycosis, meningitis are reported. The differential diagnosis and treatment are discussed.

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PENICILLIN THERAPY IN PYOGENIC DERMATOSES*

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PYOGENIC cocci flourish in diseased epidermis. Since the first report of Roxburgh⁸ on the use of penicillin in these superficial infections, many papers have appeared. There is unanimity as to the effectiveness of penicillin in impetigo, ecthyma and furunculosis; but with syco-sis and with secondary infections in eczematous eruptions and acne, previous observers report varying degrees of success. Much uncertainty remains also as to dosage and methods.

The following observations were made on 618 soldiers. The more severe cases and others who could be relieved from duty (211 in all) were treated in a hospital, the majority (407) in a dispensary. The lesions were grouped under the following diagnoses (Table 1).

IMPETIGO. There were 52 cases of impetigo contagiosa involving head and neck and 35 cases of "bullous" or "tropical" impetigo¹ involving chiefly the covered portions of the body, especially the axillæ. The results in both forms were almost uniformly good whether treatment was topical or intramuscular. This agrees with the experience of most previous observers. The rapidity of response was striking. Many cases were much improved overnight, 9 appeared well in 3 days and the median time for clearing was 6 to 7 days.

There were apparent explanations for most of the 16 failures. Three patients

had been sensitized to penicillin, 2 of them by previous treatment. Three were treated with ointment containing only 100 units of penicillin per gram. Two had also a folliculitis of the beard, a probable source of reinfection. Others were obviously irregular in applying treatment.

The frequency of relapse was surprising. Of 35 cases recalled for follow-up after 2 weeks, 10 (28%) had developed new lesions. This would appear an indictment of penicillin therapy, but it was doubtless due to the rapidity of apparent cure which led to premature cessation of treatment. It indicates, however, the necessity of continuing treatment of neighboring as well as involved areas after apparent cure. It may prove advisable to follow penicillin with ammoniated mercury or some other antiseptic.

ECTHYMA. The ecthymatous lesions encountered varied in extent and severity. Several resulted from chigger bites, others from scabies, creeping eruption or excoriated dermatitis venenata. Few seemed characteristic of the fly-borne ecthyma² which were common among combat troops in tropical areas.

Response to local or intramuscular treatment was rapid and, considering the obstinacy of these cases to other therapy, even more impressive than in impetigo. Of the 33 cases treated, 27 (82%) cleared in a median time of 7 days; 17 were fol-

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lowed from 1 to 3 weeks after healing and only 3 (18%) had relapsed. Four of 6 additional cases who disappeared after 1 or 2 days of treatment were already much improved.

Of the 6 failures; 1 appeared sensitized although he disappeared before skin tests could be carried out. A second was treated only with 100 unit gauze and resistant strains of staphylococci were recovered from the remaining 3.

to 3 years duration. Others had developed during an attack of impetigo 1 or 2 weeks before. Some may well have been transitory infections, but some cases of only a weeks duration proved very resistant to treatment.

In several Negro patients, the bearded area was covered with keloid-like papules, a type of lesion which has been variously interpreted.⁵ The foreign body effect of embedded hairs was probably the chief

TABLE 1.—RESULTS OF PENICILLIN TREATMENT

Diagnosis	No. cases*	Duration of infection†			Cultures from lesions‡			Infection controlled§	Time in days¶			Relapses observed		Treatment, failures			Reactions to treatment
		Median	Maximum	Minimum	Hemolytic streptococcus	Sensitive staphylococcus	Resistant staphylococcus		% of cases	Median	Maximum	Minimum	Cases followed 2 weeks	Relapsed	Improved	Unimproved	
Primary infections*																	
Impetigo contagiosa	52	7	60	2	19	79	38	79	6	26	3	20	32	2	17	2	8
Impetigo bulloea	35	10	1y	1	18	82	45	86	7	17	3	15	20	0	14	0	0
Ecthyma	33	14	102	3	57	36	75	82	7	37	3	17	18	9	6	3	6
Folliculitis of beard	50	40	10y	2	13	57	54	36	15	79	3	10	20	24	26	8	14
Folliculitis elsewhere	28	14	10y	1	4	50	39	29	9	15	3	5	60	14	57	0	7
Furunculosis	21	21	1½y	2	0	73	27	80	7	41	3	10	10	5	15	0	0
Hidrosadenitis	9	14	1y	2	0	86	14	100	7	39	5	2	50	0	0	0	0
Secondary infections in																	
Dermatophytosis of feet	226	.	.	.	62	35	52	82	4	37	1	118	14	8	8	2	4
Intertrigo	15	.	.	.	33	0	92	20	12	18	4	1	0	33	27	20	20
Eczematous dermatitis	144	.	.	.	46	48	75	40	11	80	2	29	38	15	34	11	25
Acne	23	.	.	.	0	12	35	4	47	47	47	1	0	13	79	4	9

* Of the 618 patients, 18 were treated for 2 lesions of different character and each lesion was considered a separate case.

† The median, maximum and minimum duration of infection is tabulated in days, except where a "y" indicates years. It was impossible to determine the duration of secondary infections.

‡ Percentages are based on cases from whom satisfactory cultures were obtained, about 80% of the total. Two or 3 types were frequently obtained from open lesions, simultaneously or at different times during treatment.

§ The infection was recorded as "controlled" when clinical signs of pyogenic infection disappeared. In the primary infections, this record coincided with the disappearance of the lesion, except for residual erythema or desquamation.

¶ "Time" records the median, maximum and minimum days under treatment, before infection was controlled.

FOLLICULITIS OF THE BEARD. It is well known that simple follicular infections of the beard respond well to antiseptic treatment, whereas other cases in which constitutional or anatomic factors or allergy seem to play a dominant rôle are most difficult therapeutic problems. There are, however, no precise criteria by which the latter group, called sycosis barbæ, can be identified. Some have reported success with penicillin therapy in sycosis, but others only temporary benefit or complete failure. The cases of this series were of unequal severity; 8 were of from 2 months

cause of their pustules. Two patients on culture yielded only a few coagulase negative staphylococci and it is doubtful if these eruptions were caused by infection. In others pustules continued to develop after pathogenic cocci could no longer be found in their lesions. One obvious difficulty in treatment was the delivery of an effective dose of penicillin at the depth of the hair follicle. Various combinations of intramuscular and oral therapy with local applications of different strength were employed in hope of achieving success.

The results were not easy to judge on

account of the frequent persistence of scattered macules and papules after active pustulation had ceased. The percentage of cases that could be considered cured was low (Table 1) and follow up periods too short to be certain of the permanence of cure in a disease so prone to relapse.

Apparent cure in 3 to 23 days was obtained in 14 cases. These varied in severity and in duration from a few days to 1 year; 10 of them were followed for 7 to 18 days and 8 showed no evidence of relapse in this brief period.

Cultures from 11 of these successful cases were studied and showed a predominance of penicillin sensitive organisms.

	Cases
Penicillin sensitive staphylococci	3
Penicillin sensitive staphylococci and streptococci	3
Penicillin resistant staphylococci	1
Sensitive cocci and resistant cocci	2
Staphylococci of doubtful sensitivity	1
Only coagulase negative staphylococci	1

It appeared that in folliculitis caused by sensitive cocci, penicillin is effective if applied in sufficient concentration.

In 4 other cases the infection was controlled only after 5 to 11 weeks of treatment. All showed penicillin resistant staphylococci, which persisted in repeated cultures. After trial of many dosages, they finally cleared under paintings with a solution of 100,000 units per cc., plus (in 3 cases) intramuscular injection of 600,000 units a day.

Eight other cases with resistant staphylococci seemed nearly clear after similar huge doses, though occasional pustules continued to appear. Still others showed striking improvement in the 1st week and developed no conspicuous lesions as long as treatment was continued. One must doubt whether any of these patients were permanently cured, but it did appear that enormous amounts of penicillin had some inhibitory effect on these resistant staphylococci.

FOLLICULITIS OF THE TRUNK AND EXTREMITIES. The results in this group of 28 patients were surprisingly poor. Only 8 cleared up, 4 others were markedly improved and 16 made no significant prog-

ress. The results of the cultures were also surprising. These eruptions, chiefly on the buttocks, thighs and legs, or in the groin or axillæ appeared to be infections, but often responsible organisms could not be isolated. In 3 cases, some of the hairs pulled from the center of pustules gave no growth when inoculated on blood plates. Other cultures showed only 1 or 2 colonies or only coagulase negative staphylococci. From only 4 did we obtain numerous colonies of penicillin susceptible, coagulase positive staphylococci. One of these cases cleared and 2 improved.

FURUNCULOSIS. Twenty-one cases of furunculosis were treated, 5 by intra-

muscular injections, 5 by oral administration, 4 by intramuscular injections followed by oral administration and 7 by local application of ointments. They were treated for 2 distinct purposes: the control of the acute furuncles and the prevention of recurrence. As furuncles discharge and heal spontaneously the percentage healing under penicillin is of no significance, but definite impressions were gained from our observation of these cases.

Local application had no effect except perhaps to accelerate healing of already discharging furuncles. The effect of intramuscular injections was dramatic in 1 case with multiple large furuncles and in 6 others appeared to hasten the involution of acute lesions. Oral treatment gave similar, though less convincing results. Relapses occurred in only 1 of the 10 cases which were followed.

HIDROSADENITIS. Uniform, at least temporary success was obtained in 9 patients with axillary lesions probably originating in the sweat glands. The majority had recent infections, but 1 had had repeated lesions for a year and another for 6 weeks. They received intramuscular injections for from 2 to 10 days, and in

3 cases this was followed by a course of oral therapy. All cleared in a median time of 1 week. Only 2 cases could be followed and 1 of these had relapsed.

DERMATOPHYTOSIS. Among the cases treated in this series were 264 pyogenic infections of the feet, which were apparently secondary to dermatophytosis; 36 cases, who showed an eczematous reaction on the dorsa of the toes, have been included for tabulation in the group of eczematous dermatidides, because it was found that the presence of such reaction influenced the response to penicillin. Of the remaining 226 non-eczematous cases, 25 had lymphangitis or lymphadenitis, which in most arose from infected dermatophytic bullæ on the sole; 42 others had similar infected bullæ without extension of the

ing results were those in cases complicated with lymphangitis and lymphadenitis. It had previously seemed necessary to hospitalize many such cases, but 19 of the 25 cleared up when bandaged with penicillin gauze and simply relieved from active duty.

These results would lead one to conclude that this antibiotic is of outstanding value in these pyogenic infections of the feet; but the bacteriologic findings make it difficult to attribute all cures to the penicillin. Of 165 cases from which cultures were studied, 90 (54%) yielded staphylococci resistant to penicillin *in vitro*. These were sometimes in pure culture, sometimes mixed with penicillin sensitive strains and often with streptococci (see table).

	No. cases	Infection controlled (%)
Penicillin sensitive staphylococci only	21	90
Penicillin resistant staphylococci only	26	73
Penicillin resistant staphylococci with or without sensitive staphylococci and/or hemolytic streptococci	90	78

infection to the lymphatics. There was a non-suppurative cellulitis of the toes in 22 cases. In 78 cases there were weeping areas where the stratum corneum was denuded and 13 showed fissures between or beneath the toes. Of the remaining 46, many showed pustules or other evidence of pyogenic infection, but some were simple intertrigos of the toes, in which it was desired to test the effect of penicillin. Fungi were demonstrated in 73% of 240 cases, scrapings from whom were examined microscopically.

The pyogenic infection appeared controlled in 82% of these cases in a median time of 4 days. The relapse rate 14% was not high. Fissures healed promptly in all 13 cases treated. Denuded areas healed in 78% of such cases in a median time of only 3 days. Cellulitis cleared in the same percentage. Two patients with a streptococcus infection, which spread by undermining the horny layer of the sole—a type of infection it has been difficult to control with other agents—did well under penicillin. The most convince-

The following observation showed that the less severe cases would heal without the aid of an antibacterial agent. Nine patients with denuded areas or infected bullæ were treated for 1 to 8 days on 1 foot with penicillin gauze and on the other with gauze impregnated with plain carbowax. In 8, both feet healed with equal rapidity. In the 9th, the penicillin foot healed more rapidly.

It is probable that in cases showing only sensitive cocci, penicillin was effective, and that in others, with mixed cultures, its inhibition of sensitive strains was decisive in controlling the infection. The overall frequency and rapidity of cure was probably increased by the routine use of penicillin.

Comparison with cases previously treated with other antibacterial agents, indicates that penicillin was more effective. During the past 3 years we have used sulfathiazole 5% combined with tannic acid 10%, zinc peroxide 20% and zephiran 0.5% extensively in treatment of pyogenic infections of the feet. Selecting only cases

with similar lesions and only those whose feet were bandaged with carbowax gauze impregnated with the antiseptic, the results were compared with the patients similarly treated with penicillin (Table 2).

In all 3 types of cases, the percentage clearing under penicillin was higher and the average time of clearing less than with the other treatment agents. The rapidity of response to penicillin was especially striking. In 3 days infection was controlled in 40% of the cases treated with penicillin, in 20% of those treated with sulfathiazole, in 18% of those treated with zinc peroxide and only 12% of those treated with zephiran.

often undetermined, and it was not expected that penicillin would influence it. On culture, however, these cases often yield a growth of coagulase positive staphylococci, more profuse than one obtains from a boil, and it was hoped penicillin would control this secondary infection which seemed frequently the chief cause of symptoms. In this series it appeared promptly effective in only 28 cases in which evidence of pyogenic infection disappeared in from 3 days to 2 weeks. These included 11 cases of probable dermatophytosis with secondary infection and eczematization, 5 eczematous eruptions on the hands and 5 on the lower

TABLE 2.—SECONDARY INFECTIONS IN DERMATOPHYTOSIS

Types of lesions	No. cases	Infection controlled (%)	Average time (days)
Infected bullæ:			
Penicillin	40	85	4
Sulfathiazole	8	62	4½
Zephiran	6	83	3
Denudation:			
Penicillin	78	81	3
Sulfathiazole	61	58	6
Zinc peroxide	28	55	11
Zephiran	8	50	5
Fissures:			
Penicillin	13	100	3
Sulfathiazole	15	67	4
Zinc peroxide	8	75	6
Zephiran	7	57	5

INTERTRIGO. There were 15 patients with intertrigo of the groin or axillæ treated with penicillin ointments; 8 were examined for fungi and all were negative; infection was controlled in only 3 cases; 5 were materially improved. It is doubtful if even the few cures obtained were due to specific action of the penicillin as out of 12 cases from whom cultures were obtained, 11 showed resistant staphylococci.

ECZEMATOUS DERMATITIS. Under this heading are included 144 heterogeneous cases all of which showed a dermatitis characterized by edema, and pin-point vesicles or areas of exudation. Most showed also pustulation or other evidence of secondary infection. The etiology of the primary dermatitis was varied and

legs and ankles which may have been primarily dermatophytids. There were 4 cases of contact dermatitis in which protection from the respective allergen doubtless aided in curing the infection. The effect of penicillin was most convincing in 3 cases of infectious eczematoid dermatitis of the toes secondary to paronychia which cleared up promptly and completely.

There were also 10 cases of chronic dermatitis of the hands, feet or lower legs from 1 month to 7 years in duration in which not only suppuration, but practically all signs of dermatitis cleared up under prolonged treatment with penicillin. Two were sensitive to shoe leather, but removal of shoes had not sufficed to clear up their infection. This small group of

therapeutic successes appeared important on account of the difficulty of aiding these chronic cases by any other procedure.

Individualization of treatment seemed necessary. Some, who reacted to strong ointment, did well under a weaker preparation. Others unimproved by weak ointments, cleared up under the use of a stronger. Some who appeared sensitized at first, could later be treated successfully after their sensitization had subsided. In several cases, the infection was finally controlled only after trying many modifications in therapy for a period of 1 or 2 months, and 1 was treated 11 weeks. Two patients who had repeatedly reacted to attempts at local therapy finally cleared up without evidence of sensitization under intramuscular injections. Conversely, 1 patient who reacted with urticaria to intramuscular injections, was successfully treated with local applications.

In only 2 out of 12 cases of seborrheic dermatitis was penicillin therapy followed by significant improvement. In a number of other eczematized cases pustulation was controlled without material relief of the patient's discomfort. In the majority, even the pyogenic infection proved resistant. This is probably explained by the frequent occurrence of penicillin resistant strains of staphylococcus and the frequency with which hypersensitivity to penicillin develops in these eczematized cases.

ACNE. Pustular or cystic acne was treated in 23 cases, again with no expectation of influencing the primary disease, but in hope of controlling the secondary infection. In only 1 case did the infection appear completely controlled. Local applications of ointment had no discernible effect in 4 cases. Intramuscular injections or oral treatment showed substantial improvement in only 3. This may well be explained by the fact that cultures from 6 cases showed resistant strains of staphylococci, and that cultures from 1 cystic case were completely sterile except for a few colonies of coagulase negative staphylococci.

The temporary successes with penicillin therapy, though few, were of some interest because incision of infected cystic lesions frequently leaves scars which are disfiguring when they occur on the face. The possibility of controlling such lesions without incision would seem a therapeutic asset, even if success is infrequent and temporary.

MISCELLANEOUS CASES. A number of patients with other dermatoses which appeared secondarily infected were treated with penicillin ointment. Among them were found 4 cases of psoriasis and 4 of lichen simplex. In 1 of these latter and 1 patient with pustular rosacea, from whom streptococci Type C were recovered, the infection cleared promptly. Most of the others from whom cultures were obtained yielded only resistant strains of staphylococci and all failed to clear under treatment.

THE SUSCEPTIBILITY TO PENICILLIN OF STRAINS ENCOUNTERED. Strains of pyogenic cocci isolated from these lesions were tested for their sensitivity to penicillin by a cylinder plate method.⁶ Most staphylococci fell into 2 groups and gave readings indicating either inhibition by 0.01 unit of penicillin or less per cc. or resistance to 100 or more units. Only a moderate number of intermediate strains were encountered. Only beta hemolytic streptococci were recorded and most were highly sensitive to penicillin. Many were of Group A, but strains of Group C and Group G were frequent and a few of Group B were isolated. The pathogenicity of the last 3 must be questioned.

Streptococci disappeared in most cases after a few days of local penicillin treatment, but occasionally reappeared in later cultures although treatment was continued. In a few cases they persisted and 1 eczema of the ankle yielded a profuse growth of penicillin sensitive streptococci (Group C) in 10 successive cultures during a 3 weeks period of local treatment. Sensitive strains of staphylococci also disappeared in most cases though with less regularity and speed. Resistant

strains of staphylococci often persisted after treatment even with concentrated solutions, but usually disappeared if the lesions healed.

The overall correlation between the response of different diseases to treatment and the sensitivity to penicillin of organisms isolated from them was good except in ecthyma and dermatophytosis. In impetigo, furunculosis and hidrosadenitis the percentages of susceptible cocci and of favorable response to treatment were high. In folliculitis, intertrigo, eczematous dermatitis and acne resistant strains were frequent and response to penicillin therapy was poor. In ecthyma and in infections secondary to dermatophytosis, however, the clinical results were excellent although the percentage of resistant strains was high. Of 14 patients with lymphangitis or lymphadenitis from whose portal lesions resistant strains of staphylococci were isolated, 11 recovered promptly under penicillin. In nearly all groups, individual cases from which resistant strains were recovered healed promptly.

Three events might account for these paradoxical results: (a) Healing may have been spontaneous or due to non-specific effects of the dressings supplied. This was probably true in many cases of dermatophytosis. (b) Inhibition of penicillin sensitive strains may have determined the cure because the coincident resistant strains were less virulent. Inhibition of the streptococci frequently present in ecthyma and dermatophytosis was probably decisive and it appeared in many cases that inhibition of sensitive staphylococci resulted in cure in spite of persistence or resistant strains. The observations of Spink,⁹ however, throw doubt on the latter hypothesis. (c) Strong ointment or large doses may have produced sufficient concentrations in some lesions to inhibit resistant strains. This could hardly be true of some strains which were viable in broth containing 1000 or 10,000 units per cc., unless one assumes that penicillin is more effective in tissue than in test tube. However, clinical observation did strongly suggest that penicillin was effective

against resistant staphylococci especially in some cases of folliculitis of the beard, which finally cleared after most intensive therapy.

SENSITIZATION TO PENICILLIN. Reactions during treatment (Table 1) or to subsequent patch or intradermal tests showed that penicillin is a potent skin sensitizer. Some form of cutaneous reaction which appeared allergic appeared in 11% of all patients and in 25% of those with eczematous dermatitis. These will be discussed in a subsequent report.³ Although many of these sensitizations were localized and transitory, they accounted for a large proportion of the treatment failures. In the brief treatment required for impetigo, ecthyma or infected dermatophytosis they seldom interfered with success but their high frequency in cases with previous eczematization seemed a warning against the local application of penicillin in such cases.

LOCAL TREATMENT. Ointments containing 100,000 and 10,000 units per gm. were freshly prepared each week in 3 types of bases, an oil in water emulsion (U. S. Army Supply Item No. 1173800), a bentonite gel and a glycol-carbowax mixture.² A solution of 100,000 units per cc. of water was painted on certain lesions. Ointments were kept on ice and reapplied every 2 hours when practicable. Gauze impregnated with penicillin in carbowax base was found convenient and economical for cases requiring dressings.

The 100 unit ointments were definitely less effective than the stronger preparations. In a few cases infection was controlled by 10,000 unit or 100,000 unit applications after the weaker ones had failed. On the other hand, sensitization developed more frequently after use of the stronger preparations. Although evidence for greater effectiveness of the stronger preparations was inconclusive, it seemed probable that ointments containing 10,000 units per gm. were advantageous in most instances and painting with 100,000 units necessary in some cases of folliculitis.

No difference in the bacteriostatic effect

of penicillin could be detected when applied in the 3 bases. The emulsion base was best tolerated by eczematous cases and carbowax proved most convenient for dressings. There is also evidence that penicillin is more stable in carbowax because it is anhydrous.⁴

INTRAMUSCULAR THERAPY. Penicillin in oil and beeswax was injected once or twice daily in doses of 300,000 units. Four cases of impetigo and 3 of ecthyma were so treated, enough to demonstrate that parenteral penicillin was effective in these superficial infections. Such treatment seemed quite unnecessary. While some cases of folliculitis of the beard did well with strong local applications, others failed to respond until given intramuscular injections. Most of our cases of lymphangitis and cellulitis responded to application of penicillin to the portal of entry, but parenteral therapy was obviously more effective. In furunculosis and hidrosadenitis, parenteral or oral, seemed the only routes of therapy worth trial. The frequency of sensitization following local treatment of eczematous dermatitis indicated that in the majority of these cases only systemic therapy should be used.

ORAL THERAPY. Penicillin was administered orally every 2 hours for 9 doses, to total 550,000 or 1,100,000 units daily. The results were inconclusive. For reasons which we could not determine, erratic and generally unsatisfactory blood levels were observed in most cases. This may have been because our patients were ambulant and on a heavy diet or possibly

because the formalin treated capsules⁷ employed in most of our cases failed to release penicillin in the upper intestine. In spite of these low blood levels, 1 impetigo, 1 folliculitis of the beard and 4 of 5 cases of furunculosis cleared after taking capsules. Oral therapy seemed effective as a sequel to intramuscular in the few cases of chronic furunculosis, folliculitis and hidrosadenitis in which prolonged follow-up treatment could be maintained. If reduced costs make the huge doses required practicable penicillin by mouth should prove useful in suppressive treatment for prevention of relapse in these chronic infections. Its use in eczematous dermatitis would greatly reduce the danger of sensitization. It may even prove more convenient than ointments in treating superficial infections such as impetigo.

Summary. 1. Local penicillin therapy was rapidly effective in the majority of cases of impetigo, ecthyma and the pyogenic complications of dermatophytosis.

2. Intramuscular penicillin was effective in furunculosis and hidrosadenitis.

3. One-third of our cases of folliculitis of the beard cleared at least temporarily under penicillin. Concentrated local applications combined with intramuscular injections often appeared necessary.

4. Secondary infections in eczematous dermatitis were controlled in 40% of cases, but sensitization developed frequently after local therapy.

5. Penicillin was seldom effective in seborrheic dermatitis, intertrigo, folliculitis of the trunk or extremities or acne.

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ELECTROCARDIOGRAPHIC PHENOMENA ASSOCIATED WITH SPONTANEOUS PNEUMOTHORAX AND MEDIASTINAL EMPHYSEMA

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In the differential diagnosis of acute, severe precordial pain, spontaneous pneumothorax alone or in association with mediastinal emphysema must be considered. The literature^{1,4,10,11,13,14} contains descriptions of a fair number of such cases and in the course of extensive hospital practice instances of spontaneous pneumothorax are not rare. There are sufficient physical signs, for the most part, to render the diagnosis readily apparent. In some instances, however, the amount of air escaping into the pleural space is small and the diagnosis can only be made by radiography. In a similar manner, although Hamman's sign is generally present in mediastinal emphysema, it often simulates a pericardial friction rub. When this is associated with electrocardiographic changes, substernal oppression and radiation of pain to the neck or to the arms, one may well consider the case to be one of myocardial infarction. Pneumothorax alone is known to produce minor changes in the electrocardiograms.^{2,8,9} These are concerned largely with rotation of the electrical axis, diminution in the height of the T waves and slight displacement of the ST segments. On the other hand, pneumothorax with mediastinal emphysema may, apparently, result in grossly abnormal electrocardiographic tracings.^{10,11} Miller¹¹ has reported a series of such cases which demonstrated a low T1, flat or inverted T4 (4F) with elevation of the ST segment and small or absent initial deflection in the 4th lead. He suggested that these findings were probably the result of the associated pneumothorax. However, he also considered the presence of a temporary functional coronary insufficiency. This was felt to result from right heart stasis inducing resistance to coronary emptying. This in turn is due to compression of the

pulmonary vessels and reduction of their lumen by air dissection of the vascular sheaths.⁸ Interference with coronary filling is also thought to occur as the result of increased mediastinal pressure. The latter would be similar in effect to increased intrapericardial pressure and could even cause tamponade of the heart. Other observers⁸ have noted that rotation of the heart on its anatomic axis may result in electrocardiographic alterations. Any or all of these factors may be operative. It is of interest to note that these cases demonstrating gross electrocardiographic changes were all associated with a left-sided pneumothorax.

It is the subject of this paper to inquire further into the causes of electrocardiographic abnormalities encountered in the presence of spontaneous pneumothorax occurring together with mediastinal emphysema.

Case Reports. CASE 1. An 18 year old soldier came into the hospital complaining of severe substernal pain which radiated to the neck and shoulders. The admission diagnosis was coronary thrombosis. The pain occurred suddenly some 2 hours earlier while patient was reaching for a telephone. It became progressively more severe and required morphine for control. The patient volunteered the information that he felt more comfortable in the erect position. The past and family history was entirely non-contributory.

When first seen the patient was pale and sweating and appeared to be in moderate shock. The pulse was 96 and of good quality. The temperature was 97.8 and the blood pressure 120/76. Examination of the lungs revealed no gross abnormalities and the heart was remarkable only in that it was percussed with difficulty and that the sounds were distant. Due to the patient's condition the examination was carried out in the supine position.

An electrocardiogram was made at once and this demonstrated a somewhat low T1, a slightly inverted T4 (4F) and an absent R4. At this time the patient was reexamined and in the erect position the characteristic mediastinal crunch was noted; the heart sounds were louder and the left border could be percussed without difficulty. A Roent-

gen ray of the chest confirmed the impression of spontaneous pneumothorax on the left side. There was an estimated 10% collapse of the lung. Lateral views were interpreted as demonstrating air in the mediastinum.

On the following day extensive electrocardiographic studies were made. These

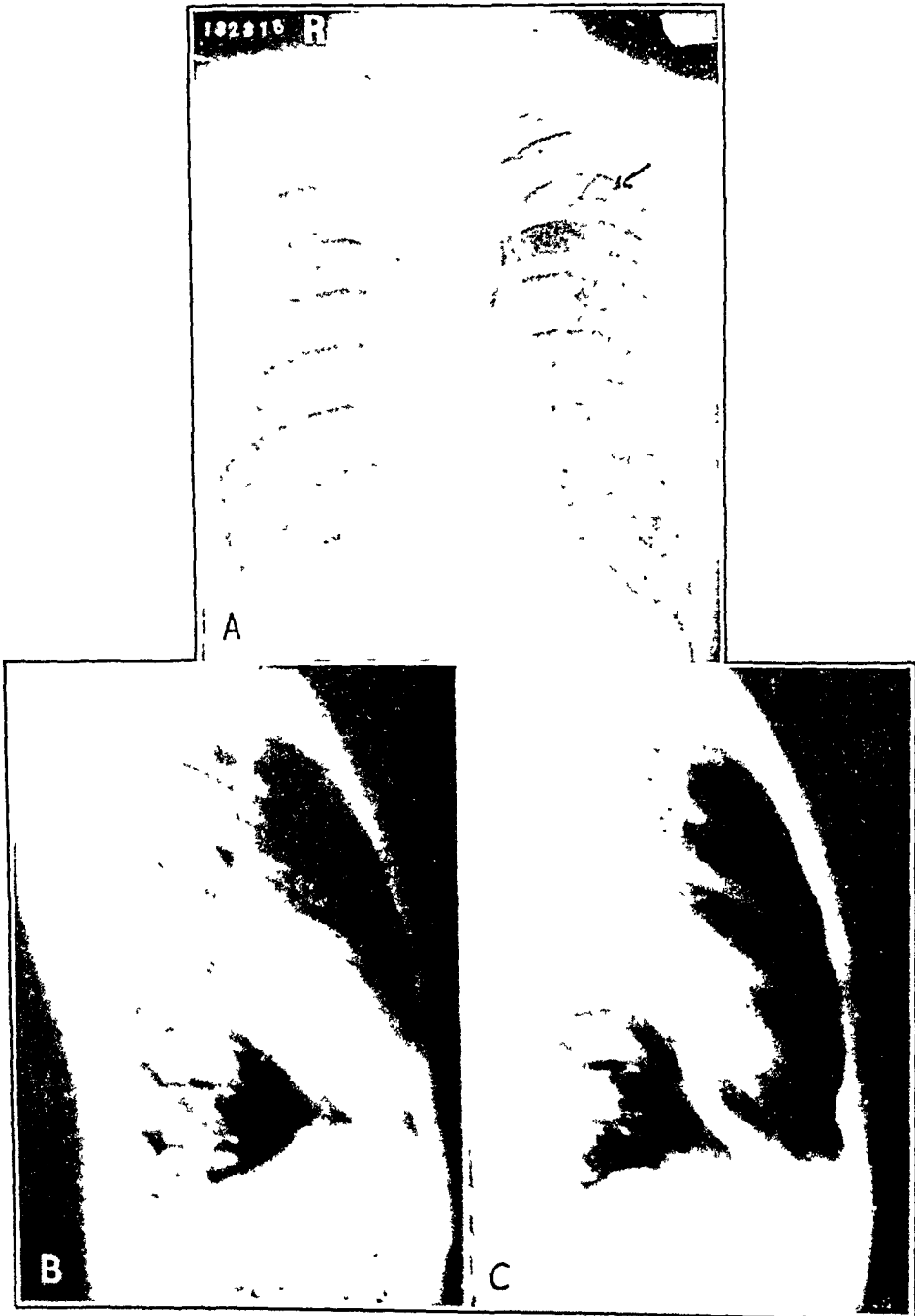


FIG. 1.—Case 1. A, Posterior-anterior view of the chest made on the day following admission. Arrow points to the partially collapsed lung. B, Left lateral erect view made on the same day. C, Left lateral view made in the supine position. Same day.

included the customary limb leads, CF2, 3 and 4, CR2, 3 and 4 in the supine, prone and erect positions, and a continuous tracing of CF3 with the patient on a radiographic tilt table. This tracing was begun with the patient in the supine position. The table was first rapidly erected, then returned to the horizontal plane. After the electro-

cardiograms were recorded, lateral radiograms were made of the chest with the patient erect and in the supine position.

It was possible to demonstrate by Roentgen ray that there was a great deal more air in the space between the sternum and the heart with the patient in the supine position than when he was erect.

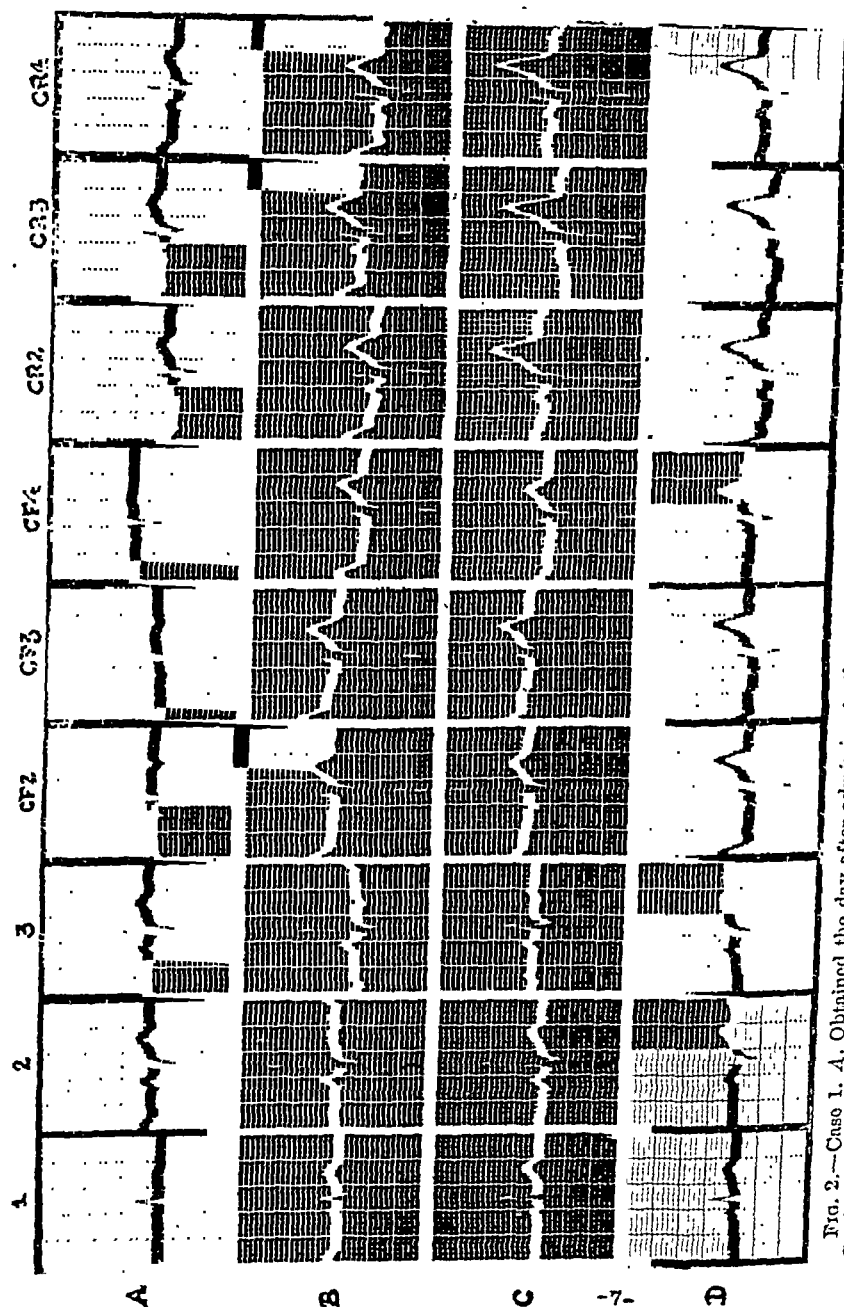


FIG. 2.—Case 1. A, Obtained the day after admission in the supine position. B, The same day; made in the erect position. C, The same day; made in the prone position. D, Made upon recovery, in the supine position.

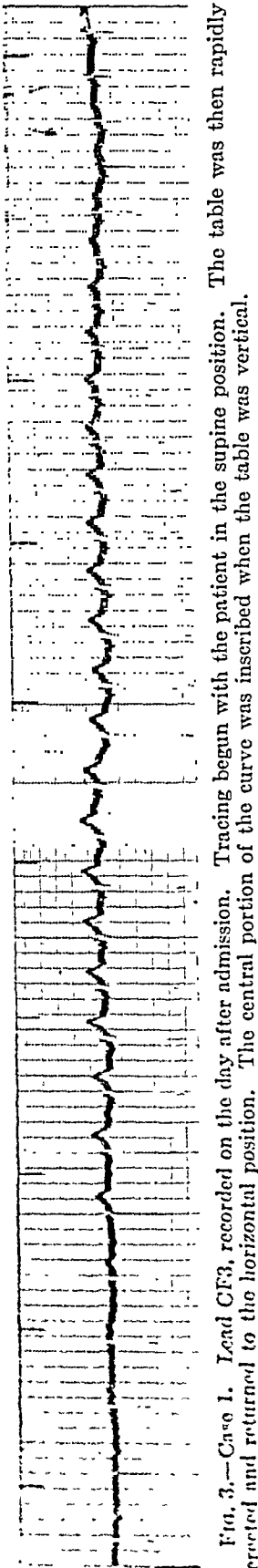


Fig. 3.—Case 1. Lead CF3, recorded on the day after admission. Tracing begun with the patient in the supine position. The table was then rapidly erected and returned to the horizontal position. The central portion of the curve was inscribed when the table was vertical.

The CF leads made with the patient supine showed T waves which were either flat or inverted; the R waves were absent or very small. The CR leads in the same position were essentially normal. T1 was low, T2 and 3 upright and S2 and 3 were prominent. In the erect and prone positions T1 was higher, T2 lower and T3 diphasic to inverted. R1 was higher and S2 and 3 very small. The T waves in the CF leads were erect although the R waves remained small. The T waves in the CR leads were upright and higher and the QRS excursions larger than in the supine position. The continuous tracing made with the patient first supine, then erect and finally supine showed a constantly altering T wave which was first inverted, then erect and finally inverted.

During the period of hospitalization which lasted 15 days the temperature went to 99.4 on several occasions and the WBC to 14,200. Except for moderate precordial discomfort during the first few days noted only in the recumbent position the patient had no complaints.

At the end of this time the mediastinal emphysema and pneumothorax had cleared entirely and the electrocardiogram became normal in all positions. The last curve still showed a rather small R wave in the CF leads but was within normal limits and essentially similar to those tracings made earlier in the erect or prone position.

CASE 2. A 19 year old soldier first experienced substernal pain and oppression approximately 2 weeks before coming into the hospital. This happened while he was working in a tank and was not associated with any severe physical effort. The pain increased in severity to the point where he had to stop his work. However, the pain receded gradually during the succeeding 3 or 4 days and he did not seek medical care. On the afternoon of his entrance into the hospital he was again working on a tank, this time lying under it, when he was taken by a sharp pain under the sternum. The pain was rather more severe than during the first attack and radiated to the left shoulder. He was subsequently admitted to the hospital. The temperature was 97.8°, the pulse 100 and the WBC was 14,300.

The diagnosis was not at once apparent and an electrocardiogram was ordered

together with other laboratory procedures. This demonstrated essentially normal limb leads. However, 4F showed a small initial deflection, elevated ST segment and inverted T wave. He was ordered back to the electrocardiograph laboratory for additional tracings and at this time the physical signs of left-sided pneumothorax with mediastinal

emphysema were noted. Roentgen rays were made of the chest and these included PA and lateral films with the patient erect and in the supine position. Electrocardiograms were made, including the customary limb leads and 4F with the patient supine, erect, prone and on his right side, 4R and a continuous 4F tracing with the patient

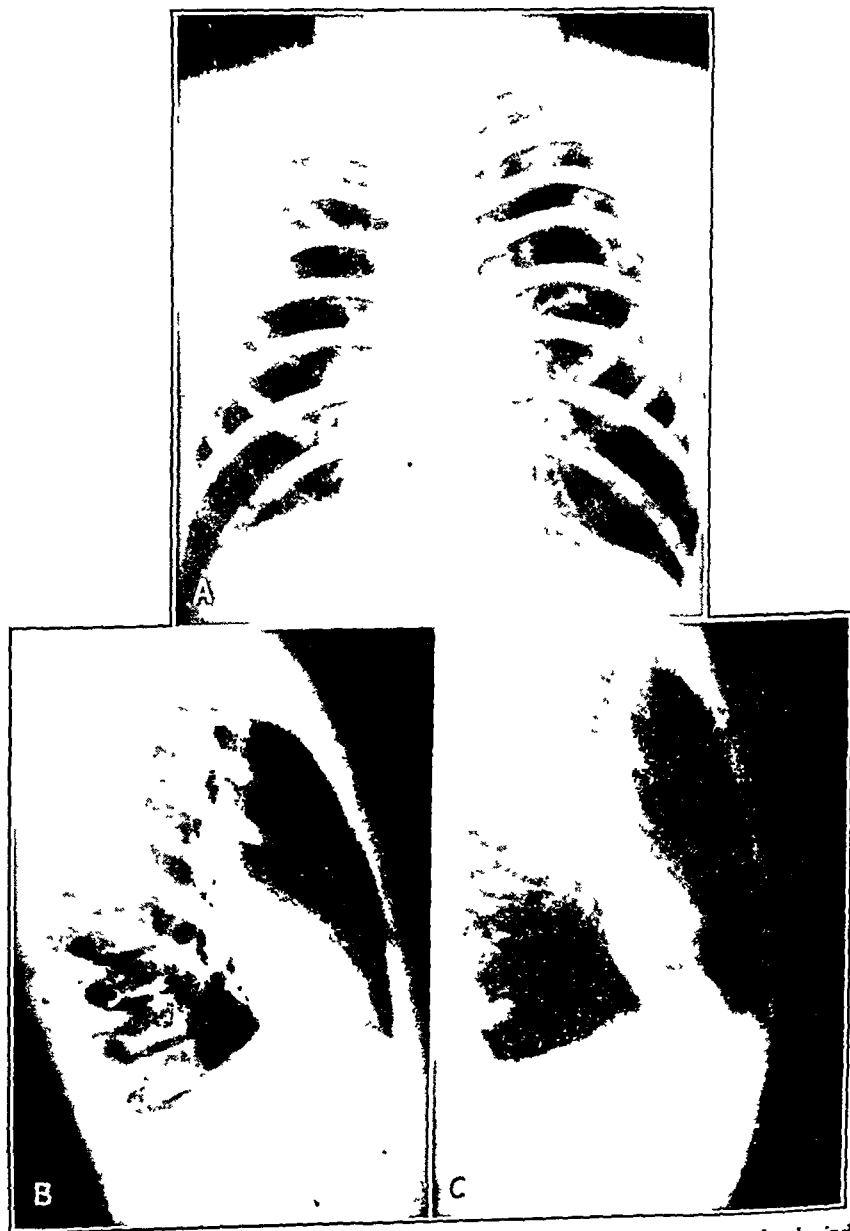


FIG. 4.—Case 2. A, A view of the chest made on admission. Pneumothorax clearly indicated B, Left lateral erect view made at the same time C, Left lateral view made in the supine position at the same time.

on a radiographic tilt table as described in Case 1.

The Roentgen rays demonstrated a left-sided pneumothorax with approximately 20% collapse of the lung. A large amount of air was seen in the retrosternal space when the patient was supine and a much smaller quantity when he was erect.

Approximately 1 week after admission the pneumothorax had diminished to approximately one-half of its original extent and the mediastinal emphysema had cleared. At this time the T wave in 4F was erect though low and did not differ materially with the position of the patient. Lead 4R was essentially unaltered.

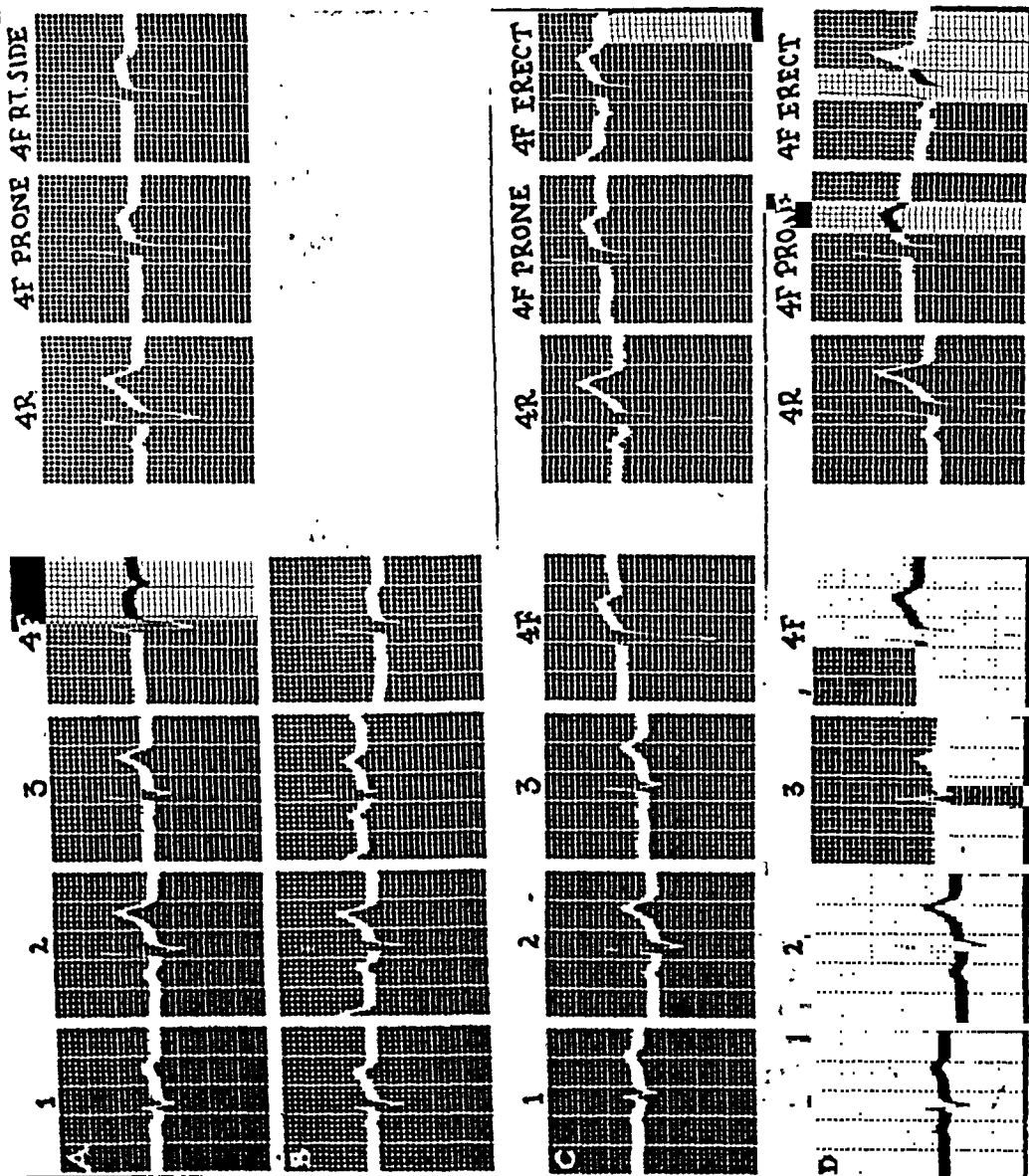


FIG. 5.—Case 2. A, Obtained on admission. All leads made in the supine position except where otherwise indicated. B, Same day. All leads made in the erect position. C, One week later. All supine except as noted. D, Upon recovery 3 weeks later. All supine except as noted.

The T wave in 4F was inverted when the patient was supine; it was erect though somewhat low when he was on his right side, prone or erect. The tilting tracing demonstrated continuous alteration of the T wave from inverted to erect to inverted. Lead 4R was entirely normal.

Some 2 weeks later (3 weeks after admission) there was complete clearing of the pneumothorax. An electrocardiogram made at that time was entirely within normal limits. The T wave in 4F was still higher when made in the erect position than in the supine. The tracing did not differ signifi-

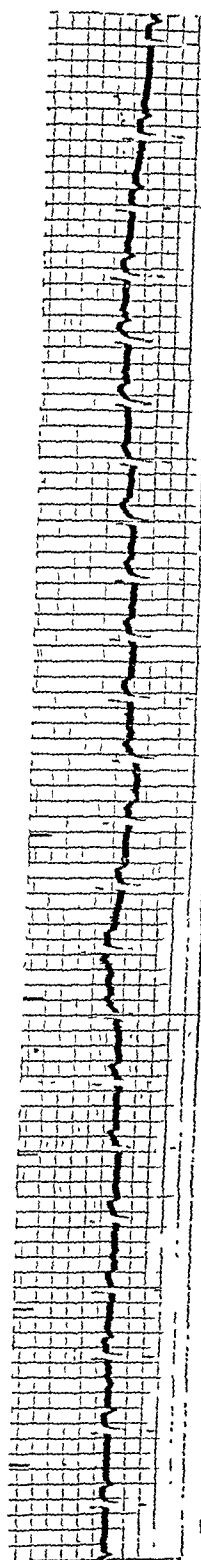


Fig. 6.—Case 2. Lead 4F, obtained on the day of admission. Tracing begun with the patient supine on a radiographic tilt table. The table was then rapidly erected and returned to the horizontal position. The first and last portions of the curve were made when the table was horizontal, the central part when it was vertical.

cantly from the one made 2 weeks earlier when some pneumothorax was still present but the mediastinal emphysema had cleared.

Discussion. In this paper we are not concerned with the pathologic physiology of spontaneous mediastinal emphysema with pneumothorax, except where it may help explain the coincidental electrocardiographic phenomena. Macklin¹ has demonstrated that air dissection of the vascular sheaths of the pulmonary vessels may occur and serve to interfere with pulmonary circulation. In support of this conclusion is the case reported by Fisher³ of a newborn infant who died with mediastinal and interstitial pulmonary emphysema. In this case the right auricle and ventricle were found to be dilated at autopsy. This interference with the pulmonary circulation is logical and understandable. However, it is difficult to see how this phenomenon would serve to impede coronary flow. In mitral stenosis with failure of the left auricle, for example, considerable increase in pulmonary pressure occurs without any interference with coronary circulation. In a similar manner, it would require a tremendous increase in mediastinal pressure to cause significant alteration in the coronary flow. Long before this took place there would be collapse of the great veins and auricles. The pain is probably better explained on the basis of dissection, tearing and splitting of pulmonary and vascular tissues than as a result of coronary inadequacy. In support of this conclusion is the almost instantaneous alteration of the electrocardiogram noted with change of position. ✓ Electrocardiographic evidence of coronary inadequacy could hardly occur so promptly or be corrected so rapidly. ✓ It is apparent from the cases reported by Miller and those noted above that extensive electrocardiographic changes may be observed in the presence of mediastinal emphysema and left-sided pneumothorax. ✓ It is of further interest were largely confined to the precordial lead and that the indifferent electrode

was placed on the left leg. The minor changes observed in the limb leads are not in themselves significant.

From the electrocardiographic data at hand certain observations appear pertinent and significant:

1. Gross electrocardiographic changes in the presence of mediastinal emphysema and left-sided pneumothorax are observed only in the CF leads.

2. These changes can be obtained only when the electrocardiogram is made with the patient in the supine position.

3. When the patient is in the prone, erect or right lateral position the tracings are essentially normal.

4. The CR leads are normal in any position although the height of the T waves and size of the QRS excursions show variations.

5. The presence of left-sided pneumothorax without mediastinal emphysema does not produce significant electrocardiographic deviations. (Case 2 at the end of 1 week with clearing of the mediastinal emphysema and persistence of the pneumothorax.)

6. Mediastinal emphysema without left-sided pneumothorax does not produce gross electrocardiographic changes. (Condition which obtains when the pneumothorax is out of the way at the apex of the chest, when patient is erect, or posteriorly when he is prone.)

7. The electrocardiographic changes found with left-sided pneumothorax and mediastinal emphysema in the CF leads when the patient is supine occur instantaneously and can be corrected in a matter of seconds by change of position.

From these observations certain conclusions are probable. It is apparently necessary to have both mediastinal emphysema and left-sided pneumothorax to produce the electrocardiographic deviations described above. Additionally, the pneumothorax must be present in front of the heart. Finally the changes are observed to a significant degree only in the CF leads. It is likely, therefore, that the observed variations are not the result

of diminution in the coronary flow but of interference with the normal distribution of the electrical impulse on the surface of the chest.

Katz and his associates^{5,6,12} have postulated that the configuration of the electrocardiogram is dependent to a large extent on the character of the tissues interposed between the heart and the electrodes. In a series of experiments during which they inserted rubber dams between the heart and its contiguous structures they were able to demonstrate marked variations in electrical potential dependent upon the position of the insulator. Stated another way, if the currents generated in the living heart produce a given curve when obtained from 2 electrodes on the surface of the body, this curve will alter if these currents are forced to complete their circuit by a different pathway. The electrodes will then be in either a more or less favorable position and the tracing will reflect this difference. In the cases under discussion this occurs when a non-conductor, air, is interposed between the myocardium and the exploring electrode. A somewhat similar explanation¹² is offered for the difference observed in the precordial electrocardiogram of young children as compared with adults. Since normally the largest portion of the electrical current of the heart is conducted away by the posterior muscle masses and the great vessels,⁶ little change is expected or found in the limb leads in the presence of mediastinal emphysema with pneumothorax. On the other hand, that portion of the current conducted to the front of the chest is seriously interfered with by the presence of air and results in the gross changes described. One must postulate further that the potentials existing between the right arm and the precordium are not interfered with to the same extent as those between the heart and the left leg. That some interference exists is indicated by the diminution in the height of the T wave in the CR leads when the patients were supine.

It is possible that if the mediastinal

emphysema were of sufficient magnitude the extensive electrocardiographic changes would be found without the simultaneous presence of left-sided pneumothorax. In the cases described, however, it is apparent that the additional insulation afforded by the pneumothorax when the air is in front of the heart is necessary to produce the alterations described. On the other hand, the pneumothorax alone cannot obliterate the precordial conduction tissue sufficiently to cause significant changes.

To reiterate, the cardiac action currents reach the chest wall largely via the connective tissue which lies between the heart and the sternum and by direct contact of the pericardium to the chest wall. When the connective tissue becomes infiltrated with air its conductivity becomes impaired and corresponds to that of lung tissue which normally is minimal.⁶ However, conduction continues to take place where the heart is in contact with the chest wall to the left of the sternum. When this, too, is obliterated by the presence of a shifting pneumothorax in the supine position, the circuit is completed by a totally different route and

results in gross alterations of the electrocardiogram. If the pneumothorax is sufficient in degree with extensive collapse of the lung the shifting phenomenon would probably not take place.

In view of the fact that the only gross abnormalities were noted in the CF leads obtained in the supine position, it would appear logical to employ other leads and positions when electrocardiographic variants are encountered.

Summary. 1. Two cases of mediastinal emphysema with spontaneous left-sided pneumothorax are described, together with the electrocardiographic changes encountered.

2. Evidence is submitted in support of the hypothesis that the electrocardiographic alterations observed are the result of the presence of air between the heart and the exploring electrode, causing interference with electrical conduction; and that the changes are not the result of impedance of the coronary circulation.

3. It is recommended that CF leads be made in various positions and that CR leads be taken.

One additional instance of left-sided spontaneous pneumothorax with mediastinal emphysema was observed since this paper was submitted. This occurred in a young man who was being studied for the late results of trench foot. The incident took place in the hospital and was not associated with physical effort. The same characteristic electrocardiographic changes were noted in the CF leads made in the supine position. Nine days later, coincident with clearing of the physical and radiographic findings, the electrocardiogram became entirely normal.

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SOME FACTORS THAT MODIFY THE EFFECT OF TRISODIUM CITRATE ON THE ABSORPTION OF ORAL PENICILLIN

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SINCE the demonstration by Free, Leonards, McCullagh and Biro⁶ that appreciable quantities of penicillin may be absorbed from the normal gastro-intestinal tract, numerous subsequent reports have confirmed the fact that the oral administration of penicillin may produce therapeutically effective blood levels. All such reports have agreed to the extent that relatively large doses of oral penicillin are necessary for the establishment of such blood levels. From the viewpoint of the quantities required, the oral administration of penicillin must therefore be considered an inefficient method of therapy as compared to parenteral injection. However, in view of the obvious advantages inherent to oral therapy, efforts have been devoted to devise means for increasing the absorption of oral penicillin. Accordingly, numerous studies have been made on the effect of various adjuvants and vehicles on the absorption of oral penicillin.

Of the many adjuvants proposed for use with oral penicillin, gastric antacids have received much attention and a number of investigators have recorded the beneficial effect of the addition of various alkalies to penicillin, whereas other reports have failed to demonstrate any marked advantage in their use. György *et al.*⁷ working largely with children and infants, concluded that the simultaneous administration of trisodium citrate led to more prolonged and higher blood levels than when penicillin was administered alone. Charney, Alburn and Bernhart⁸ substantially confirmed the conclusions of György and his collaborators, but noted a some-

what lesser effect. Cutting, Halpern, Sultan, Armstrong and Collins⁴ have also demonstrated the beneficial effect of alkalies. Other reports have noted a similar effect of aluminum hydroxide.¹³ On the other hand, such reports as those by McDermott *et al.*¹⁰ and Finland, Meads and Ory⁵ failed to demonstrate that the addition of alkalies markedly affected the extent of absorption. Numerous other uncited reports have confirmed one or the other of the two groups of investigators. Thus, although many reports have been published concerning the oral administration of penicillin, various claims remain unconfirmed and there still exists a marked disagreement concerning the effect of antacids.

Unfortunately, it has not been possible to establish a valid basis for the comparison of such results as those cited above inasmuch as the administration of oral penicillin has not always been investigated under comparable conditions. Thus, whereas György *et al.*⁷ determined absorption in infants and children, others^{4,5,10} worked principally with adults. The size of the individual doses administered has also varied from 20,000^{3,7} to 315,000¹⁰ units. Certain groups^{4,7,10} have reported the extent of absorption primarily in fasting subjects, others^{3,5} have investigated in detail absorption in both the fasting and the post-absorptive state. Finland, Meads and Ory⁵ reported results obtained from the use of some proprietary preparations, the nature of which has been kept a secret.

In a preliminary communication,¹¹ we investigated some of the factors that appeared to influence the absorption of

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oral penicillin by normal adults. Our results indicated that both the size of the dose and its relation to mealtime may have an important influence on the effect of trisodium citrate on the absorption of oral penicillin. These results are reported here in the hope that they may be useful in reconciling some of the inconsistencies noted in previous investigations.

Methods. Young, healthy, adult, male volunteers were given different dosages of penicillin either before or after mealtime, usually breakfast, and blood levels and, in many instances, total urinary excretions were determined at intervals subsequent to its administration. Calcium penicillin plus trisodium citrate was given in the form of tablets, each tablet containing 1 gm. of citrate plus 25,000 units of calcium penicillin. Calcium penicillin "without buffer" was administered in ordinary gelatin capsules, each capsule containing 50,000 units intimately mixed with lactose as a filler.

Penicillin was determined in both serum and urine by a slight modification of the serial-dilution method of Hobby, Meyer and Chaffee.⁶ All determinations were made in conjunction with duplicate standards of calcium penicillin with the C203MV strain of hemolytic streptococcus as the test organism. In the conditions used, this organism was sensitive to approximately 0.01 units per ml. when the presence or absence of grossly visible growth after 24 hour incubation was used as the sole indicator.

Following ingestion of the penicillin, all subjects were permitted to perform their ordinary duties during the intervals between assays and were not cautioned in regard to restriction of fluids.

Results. Since our original report, our experience has been increased to include the results of blood and urine assays performed on over 150 subjects. One subject who ingested 175,000 units of penicillin plus 7 gm. of trisodium citrate on a fasting stomach became temporarily nauseated. None of the other subjects complained of any ill-effects from any of the preparations used.

For the sake of clarity the results are summarized under a number of different headings. In deference to brevity, repre-

sentative results are presented rather than a detailed list of all assays performed.

1. **CALCIUM PENICILLIN** ("Without Antacid"). (a) *One Hour After Breakfast.* The results in Table 1 demonstrate that the administration of 100,000 units 1 hour before breakfast generally resulted in appreciable blood levels for 2 hours and in the recovery of appreciable but variable amounts in the urine over a 12 hour period. The ingestion of 50,000 units resulted in appreciable but usually markedly decreased urinary excretions. Less than 0.0125 units per ml. were generally present in the serum. Our failure to produce satisfactory blood levels by administration of 50,000 units was somewhat disappointing but not entirely unexpected since others⁴ have also noted that "It is probable that there would be many failures with single doses of 50,000 units of penicillin."

(b) *One Hour After Breakfast.* The results in Table 2 illustrate the fact that the administration of either 50,000 or 100,000 units usually resulted in negative blood levels and relatively insignificant urinary excretions.

2. **CALCIUM PENICILLIN WITH TRISODIUM CITRATE.** (a) *One Hour Before Breakfast.* The results in Table 3 illustrate the satisfactory blood levels that were found for 2 to 3 hours after the administration of either 50,000 or 100,000 units. The same results indicate high percentages of urinary recovery but subjects with similar blood levels frequently were found to excrete markedly divergent amounts of penicillin.

(b) *One Hour After Breakfast.* The results of Table 4 indicate that, in general, both the blood levels and the urinary excretions were similar to those obtained when penicillin was administered to the fasting subject.

Discussion. The importance of the size of the individual dose as a determinant of the effect of trisodium citrate on the absorption of oral penicillin does not appear to have received adequate emphasis. In general, the blood concentrations obtained after administrations of various quantities

of penicillin increase with the size of the dose. However, this increase is not directly proportional to the dose, successive increases of the latter producing decreasing increments in the height of the blood level.⁴

If trisodium citrate protects penicillin against destruction by the gastric acid, the administration of a given dose of penicillin with trisodium citrate would, in effect, be equivalent to administration of a somewhat larger dose of penicillin alone.

TABLE 1.—ORAL ADMINISTRATION OF CALCIUM PENICILLIN, 1 HOUR BEFORE BREAKFAST

Dosage (units)	Serum levels in units penicillin per ml.				Total units penicillin recovered in urine over 12 hours
	Hours after administration				
	1	2	3	4	
100,000	0 2	0 025	0	0	26,524
100,000	0 2	0 025	0	0	23,494
100,000	0 2	0 05	0	0	42,953
100,000	0 1	0 025	0	0	6,984
100,000	0 2	0 0125	0	0	5,881
100,000	0 1	0.05	0 025	0	*
50,000	0 025	0	0	0	6,573
50,000	0	0	0	0	1,290
50,000	0 025	0	0	0	13,832
50,000	0 025	0.025	0	0	14,772
50,000	0	0	0	0	2,088
50,000	0	0	0	0	*

* Not estimated.

TABLE 2.—ORAL ADMINISTRATION OF CALCIUM PENICILLIN 1 HOUR AFTER BREAKFAST

Dosage (units)	Serum levels in units penicillin per ml.				Total units penicillin recovered in urine over 12 hours
	Hours after administration				
	1	2	3	4	
100,000	0	0	0	0	2753
100,000	0	0	0	0	1597
100,000	0	0	0	0	1983
100,000	0	0 0125	0	0	1134
100,000	0	0	0	0	*
100,000	0	0	0	0	*
50,000	0	0	0	0	865
50,000	0	0	0	0	1019
50,000	0	0	0	0	*
50,000	0 0125	0	0	0	*
50,000	0 0125	0.0125	0	0	*

* Not estimated.

TABLE 3.—ORAL ADMINISTRATION OF CALCIUM PENICILLIN WITH TRISODIUM CITRATE
1 HOUR BEFORE BREAKFAST

Dosage (units)	Serum levels in units penicillin per ml.				Total units penicillin recovered in urine over 12 hours
	Hours after administration				
	1	2	3	4	
100,000	0 4	0 05	0 025	0 0125	23,680
100,000	0 2	0 1	0 05	0 0125	42,748
100,000	0 2	0 025	0	0	5,302
100,000	0 4	0 1	0	0	30,076
100,000	0 4	0 05	0 025	0	41,502
50,000	0 05	0 05	0	0	16,919
50,000	0 2	0 1	0 0125	0	13,628
50,000	0 1	0 025	0	0	8,797
50,000	0 2	0 025	0	0	*
50,000	0 1	0 05	0	0	*

* Not estimated.

It would follow that the effect of trisodium citrate should be most marked when given with smaller doses of penicillin. Actually, such seems to be the case. A marked effect of trisodium citrate on the absorption of 20,000 to 40,000 units has been described.^{3,7} Our results and those of others⁴ demonstrate a similar action with 50,000 units. The results in Tables 1 and 3 and those of Finland, Meads and Ory⁵ show little, if any, effect after 90,000 to 100,000 units and no effect has been found with still larger doses.¹⁰ It is therefore attractive to postulate that many of the apparent inconsistencies cited above in regard to the effect of the simultaneous administration of penicillin with trisodium citrate disappear when due consideration is given to the size of the dose.

strated that such was the case and their observations have been confirmed by others^{3,5} and by the results in Tables 1 and 2. Furthermore, the charts of Finland, Meads and Ory⁵ demonstrate that in patients with pernicious anemia, in whom the ingestion of food fails to stimulate acid production, there is less interference in the absorption of penicillin after mealtime than in normal subjects.

We believe that this effect of the previous ingestion of food is, in fact, in part due to the destruction of a large proportion of the penicillin so that, in reality, the quantity of effective penicillin available for absorption represents but a small fraction of the quantity actually given. In other words, the administration of 100,000 units after mealtime is presumed

TABLE 4.—ORAL ADMINISTRATION OF CALCIUM PENICILLIN WITH TRISODIUM CITRATE
1 HOUR AFTER BREAKFAST

Dosage (units)	Serum levels in units penicillin per ml.				Total units penicillin recovered in urine over 12 hours
	Hours after administration				
	1	2	3	4	
100,000	0 4	0 1	0 025	0 025	37,000
100,000	0 8	0 1	0	0	36,000
100,000	0 2	0 1	0.0125	0	29,400
100,000	0 025	0 025	0	0	14,514
50,000	0 1	0 1	0 05	0	15,592
50,000	0 1	0 1	0	0	17,800
50,000	0 4	0 05	0	0	26,090
50,000	0 025	0	0	0	3,226

The original demonstration by Rammelkamp and Helm¹² that penicillin was readily absorbed from the gastro-intestinal tract of achlorhydric patients has been repeatedly confirmed.^{4,5} Accordingly, there is a theoretical justification for the inclusion of an antacid in preparations of oral penicillin. However, if trisodium citrate is effective by virtue of its tendency to protect penicillin from destruction by the gastric juice, it should be most effective under conditions in which there is a maximum interference with the absorption of penicillin as a result of the increased production of acid. Inasmuch as gastric secretion is stimulated by the ingestion of food, minimal absorption of penicillin might be anticipated to occur in the postprandial state. György *et al.*⁷ demon-

equivalent to the administration of, say, 30,000 units to a fasting subject. If, however, the addition of trisodium citrate could prevent the destruction of such a large proportion of the penicillin, the postprandial administration of 100,000 units with trisodium citrate might be expected to give results similar to those obtained after ingestion of approximately the same quantity by a fasting subject. Charney, Alburn and Bernhart³ first demonstrated this decidedly beneficial effect of trisodium citrate after meal time. Our results in Tables 1, 2, 3 and 4 are in complete agreement and indicate that the theoretical deductions concerning the effect of trisodium citrate on the postprandial absorption of penicillin conform to the experimental observations. We are at a loss to

explain the findings of Finland, Meads and Ory⁵ who concluded that no beneficial effect was obtained by the use of "buffers." However, even in their experience, aluminum hydroxide gel gave somewhat better results after mealtime.

Just as is the case in the administration of penicillin to the fasting subject, we anticipate that the effect of trisodium citrate will become decreasingly evident as the size of the postprandial dose is progressively increased. Indeed, a few trials with 500,000 units have given evidence of such a decreasing effect but such experiments have been too few to warrant definite conclusions.

However, we have been unable to demonstrate that the oral administration of penicillin markedly prolongs the maintenance of effective blood levels. Indeed, analysis of the rates of excretion in Table 5 indicates that although the antacid may influence the total quantity of penicillin absorbed, such a quantity is rapidly excreted from the body. These conclusions are supported by the figures for the blood assays, since, in general, blood levels tended to fall below therapeutically effective values in 3 hours or less.

These results indicate the necessity for the frequent repetition of oral doses for the maintenance of effective blood levels

TABLE 5.—RATES OF EXCRETION OF PENICILLIN ADMINISTERED BY MOUTH

		% penicillin (cumulative) of total excreted in urine over 12 hours				
Dosage (units)	Total units penicillin excreted in urine over 12 hours	Hours after administration				
		2	4	6	8	10
<i>With citrate</i>						
1 hour before meal						
100,000	9,005	42.6	93.8	98.0	99.3	99.9
100,000	43,198	59.0	94.6	98.7	99.8	100.0
50,000	8,797	51.7	94.6	99.6	99.8	100.0
50,000	16,949	93.6	97.0	99.1	99.6	100.0
1 hour after meal						
100,000	14,514	57.3	94.8	97.9	99.5	100.0
100,000	29,400	65.3	95.8	98.8	99.7	100.0
50,000	3,226	71.4	87.8	97.6	99.5	100.0
50,000	27,930	50.0	81.2	97.0	99.7	100.0
<i>Without citrate</i>						
1 hour before meal						
100,000	6,984	69.6	93.0	98.7	99.7	100.0
100,000	42,953	85.8	94.7	99.5	99.9	100.0
50,000	1,290	90.5	99.9	100.0	100.0	100.0
50,000	6,573	82.8	99.8	100.0	100.0	100.0
1 hour after meal						
100,000	1,134	70.5	85.3	96.9	100.0	100.0
100,000	2,753	87.2	98.0	99.8	100.0	100.0
50,000	486	62.2	96.9	99.9	100.0	100.0
50,000	1,019	68.7	88.7	98.3	99.9	100.0

The arguments and facts presented above should not be interpreted as a proof of any decided advantage of trisodium citrate over other antacids for we believe that any effective non-toxic antacid will produce similar effects. Cutting *et al.*⁴ noted no marked differences between the activities of 3 such alkalies and other reports have established the advantages of various other antacids.^{9, 12}

just as in the case of parenteral therapy. Such a consideration emphasizes anew the advantage of the use of an antacid since oral penicillin therapy should conceivably be continued throughout the day without interference by meals. The principal present objection to the widespread use of oral penicillin therapy resides in the greater quantities required for successful treatment. The results of György *et al.*¹³

and Charney, Alburn and Bernhart,³ as well as those in this report, indicate that smaller quantities of penicillin may be required to overcome the interference of eating if used in conjunction with an antacid. Thus, frequent repeated doses may be given safely without regard to meal-time. There is little doubt that larger doses may be effective without an alkali and Bunn, McDermott, Hadley and Carter² have reported on the successful treatment of pneumonia by the oral administration of penicillin without an added antacid. However, they felt constrained to employ 5 times as large a dosage as that required for intramuscular therapy. In contrast, we have reported¹ the successful treatment of gonorrhea by oral penicillin plus trisodium citrate and have used only $2\frac{1}{2}$ times the total dosage required by the Army for treatment by intramuscular therapy. Obviously, any factors which tend to decrease the differential between the total dosages of penicillin required for intramuscular and oral therapy would tend to operate in favor of adoption of the latter as the method of choice.

Summary and Conclusions. 1. The influence of the size of the dose, its relation to meal time and the effect of trisodium citrate on the absorption of oral

penicillin were investigated in a series of normal adult subjects.

2. Appreciable absorption of calcium penicillin occurred in fasting subjects given a dose of 100,000 units but not with a dose of 50,000 units. Neither dose resulted in appreciable absorption when given 1 hour after breakfast.

3. In fasting subjects, the addition of trisodium citrate to calcium penicillin markedly increased the absorption of 50,000 units but the effect on the absorption of 100,000 units was less pronounced. In subjects given calcium penicillin 1 hour after breakfast, the addition of trisodium citrate markedly increased the absorption of both 50,000 and 100,000 units.

4. Although the various factors influenced the total quantity of penicillin absorbed, they did not appear to affect the rate of its excretion from the body.

5. Statements concerning the effect of antacids on the absorption of oral penicillin should be qualified by consideration of the dose and its relation to eating. It is concluded that the effect of trisodium citrate and of eating becomes less pronounced with progressive increase in the size of the dose and that the maximum advantage of the inclusion of trisodium citrate in a dose of oral penicillin is observed after administration of smaller doses.

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EFFECT OF ANTIMONY ON THE ELECTROCARDIOGRAM

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TRIVALENT and pentavalent antimony compounds have been used extensively in the treatment of human schistosomiasis and leishmaniasis and more recently to a limited degree in human filariasis. The major application of antimony as a therapeutic agent in the recent war has been its use in the treatment of schistosomiasis japonica. Fuadin and tartar emetic, both trivalent antimonials, are the drugs principally used in the treatment of this infection. Pentavalent antimony compounds (neostam, neostibosan and stibanose) have had only limited application during the war to a relatively small number of cases of leishmaniasis and to a less degree in the experimental treatment of filariasis. It is anticipated that a number of individuals with schistosomiasis will require retreatment subsequent to their release from military service and such treatment will be given in private practice or at veteran facilities. It is therefore our purpose in this paper to report certain changes in the electrocardiogram which have been observed during treatment of schistosomiasis patients at this hospital with tartar emetic and fuadin.

In 1939 Mainzer and Krause,⁵ in attempting to explain sudden deaths which occur during antimony therapy, made a study of electrocardiographic changes in 12 schistosomiasis patients during treatment with tartar emetic. They found ST-interval and T-wave changes which were "definitely pathological" in 3 cases, suspicious in 4 cases, and insignificant in 2 cases. They believed these changes to be due to the direct effect of antimony on the myocardium, and explained cases of sudden death on the bases of an exag-

geration of this same toxic action resulting ultimately in auricular (?) fibrillation and death. Tarr⁷ recently reported T-wave changes in approximately 80% of 66 patients during treatment with antimony for infections with *Schistosoma japonicum*. These changes, consisting in lowering or inversion of T waves in all leads, occurred in 100% of the patients receiving tartar emetic and in 53% of those receiving fuadin.

This paper concerns itself with an analysis of electrocardiograms taken on 100 patients during treatment with tartar emetic and fuadin. These patients were all observed and treated at Moore General Hospital, between April and December 1945 for infections with *S. japonicum* and *S. mansoni*. In none of these cases was there any clinical evidence of cardiovascular disease either before, during or after therapy, nor did any fatality occur.

Methods. Patients in this study were treated with either fuadin (a trivalent antimony compound given intramuscularly in 6.4% solution) or tartar emetic (also a trivalent antimony compound given intravenously in freshly prepared 0.5% solution in 10% glucose). The following treatment schedules were used:

1. *Fuadin 70 cc.* The entire course of treatment lasted 27 days. Patients received 1.5, 3.5 and 5 cc. on Days 1, 2 and 3, respectively. Then 5 cc. were given intramuscularly every other day for 12 injections until a total of 70 cc. had been given. Total amount of fuadin was 4.48 gm., representing 0.599 gm. of antimony.

2. *Fuadin 100 cc.* The entire course of treatment lasted 14 days, 2, 4 and 6 cc. being given on Days 1, 2 and 3, respectively, and then 8 cc. intramuscularly every day

for 11 injections until a total of 100 cc. had been given. Total amount of fuadin was 6.4 gm., representing 0.870 gm. of antimony.

3. *Tar Emetic 1.45 gm.* The entire course of treatment lasted 31 days, 5, 10 and 15 cc. of 0.5 solution being given on Days 1, 3 and 5, and then 20 cc. intravenously every other day for 13 injections, until a total of 290 cc. had been given. Total amount of tartar emetic was 1.45 gm., representing 0.522 gm. of antimony.

4. *Tartar Emetic 1.8 gm.* The entire course of treatment lasted 29 days, with 8, 12, 16, 20 and 24 cc. of 0.5% solution given on Days 1, 3, 5, 7 and 9, respectively, and then 28 cc. intravenously every other day for 10 injections, until a total of 360 cc. had been given. Total amount of tartar emetic was 1.8 gm., representing 0.648 gm. of antimony.

Electrocardiograms were taken before and at various times during and after treatment. This study is based on a total of 315 such records taken on 100 patients who had received one of the above courses of antimony therapy as treatment for schistosomiasis. Some of the patients had only 2 cardiograms taken, i. e., a control and a record taken during or immediately after antimony therapy while others had more frequent observations. Unfortunately, in many cases the maximum effect of therapy was not recorded because electrocardiograms either were taken too early in the course of treatment or, in some cases, were taken several days after the completion of antimony.

All records were taken with the patient in the supine position. The standard limb leads and CF_4 were used. Records were carefully standardized so that 1 mv. caused a deflection of 10 mm. When actual measurement revealed this distance to be less than 9 or more than 11 mm. the record was excluded from this study. In this paper electrocardiographic changes will be described in the accepted terms for various electrical components and time intervals as advocated in "Nomenclature and Criteria for Diagnosis of Diseases of the Heart."⁶

Results. *The Heart Rate.* No changes were observed in the heart rates of patients during antimony therapy. The mean heart rate in the 100 control records was 78.2 per minute as compared to 78.7 per minute during treatment.

P Waves. Many of our patients showed the commonly seen variations in amplitude and contour of P_3 , usually with the respiratory cycle. These changes, being within normal limits, were disregarded. However, in 11% of the patients a definite increase in the amplitude of P waves in Leads 2 and 3 occurred during antimony therapy. In addition, the contour of P_2 and P_3 was altered from smooth rounded curves to tall, sharp, peaked waves. In some cases the increase in amplitude in Lead 3 resulted in an inverted or diphasic P wave becoming tall, upright and peaked. No change was noted in the actual duration in time of the P deflection. Records illustrating these changes are shown in Figures 1, 2 and 3.

Of the 11 cases showing these P-wave changes, 9 showed alteration of both P_2 and P_3 , while in the remaining patients Lead 2 alone was involved. No significant changes were noted in P waves in Leads 1 and 4. Generally speaking, records showing the most marked alterations in P waves also showed considerable change in T waves which will be described later. However, numerous records showing pronounced effect of antimony on T waves showed no change in P waves from control levels.

The duration of this abnormality is difficult to determine in this study because of an insufficient number of records taken after completion of therapy. However, records on 1 patient show the P waves to be still altered 15 days after therapy and to have returned to control levels 40 days after treatment. In other cases showing P-wave changes during antimony treatment, records taken 40, 50 and 60 days after therapy revealed P waves identical with those in control records. In still another patient P_2 and P_3 had shown little tendency to return to control levels 56 days after the last injection. From these rather spotty observations no definite conclusions as to duration of P-wave changes after cessation of therapy can be drawn. However, it is apparent that in some patients these changes may

persist for as long as 2 months after completion of therapy with antimony.

PR Interval. No change in PR interval was found during antimony therapy. The mean PR interval before therapy was 0.148 second as compared to 0.1492 second during treatment.

QRS Complex. The antimony therapy caused no change in contour or amplitude of QRS complexes. In all cases the duration of QRS was within normal limits

both before and during antimony therapy. No changes in electrical axis were noted.

ST Segment. In 7% of our patients it was noted that slight elevation or depression of the ST segments, not previously observed in the control records, occurred during antimony therapy. In all cases these changes were very slight in degree, approximating 1 mm., and followed no specific pattern as to which leads were involved. ST₁ and ST₂ were elevated on

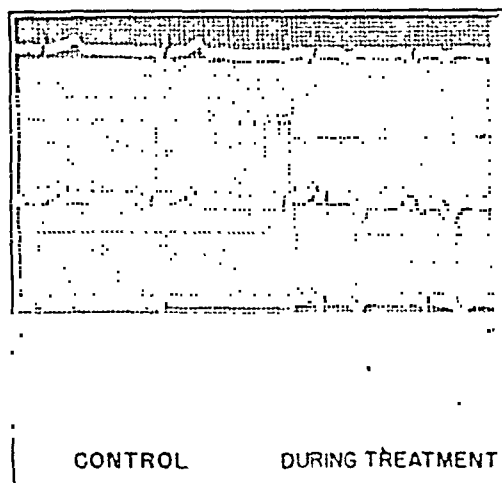


FIG. 1.—Electrocardiographic changes during administration of tartar emetic. "Control" record before treatment. "During Treatment" record taken on the 32nd day of therapy 24 hours after injection of 20 cc. of 0.5% solution. Total dose prior to this record was 290 cc. of 0.5% solution tartar emetic = 1.45 gm. = 0.522 gm. antimony. Note increase in height in P₂ and P₃ and inversion of all T waves. QT interval prolonged. Record taken 50 days after completion of treatment showed disappearance of abnormalities noted during treatment.

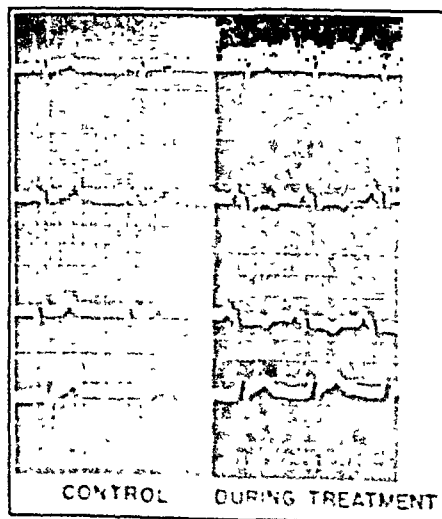


FIG. 2.—Electrocardiographic changes during administration of tartar emetic. "Control" record prior to treatment. "During Treatment" record taken 48 hours after last dose of 20 cc. of 0.5% solution tartar emetic. Total course of treatment consisted of 1.45 gm. (see text for schedule of administration). Note increase in P₂ and P₃ and decreased amplitude of T wave in Lead I and inversion of Leads 2 and 3.

2 records, depressed in none; ST_3 was never elevated but was depressed in 2 records; and ST_4 was elevated on 5 occasions and never depressed.

A more common change involving the ST segment and occurring in 45% of the patients during antimony therapy consisted of fusion of the ST segment with the T wave so that the 2 were very difficult to separate. In many normal electrocardiograms it is impossible to find a definite point where the ST segment ends and the T wave begins, so that the determination of which records in this study showed ST-

T wave but in many records there appears to be an actual change in the ST segment, as well, producing a single smooth curve.

In some cases this abnormality was transient in nature, occurring fairly early in treatment and disappearing later in the course of antimony therapy when T-wave changes became more marked. The relative frequency of occurrence of ST-T fusion in different leads was as follows: Lead 1, 55.6%; Leads 2 and 4, 53.4%; Lead 3, 37.8%. (These figures indicate percentage of the entire number of cases showing this abnormality.) In

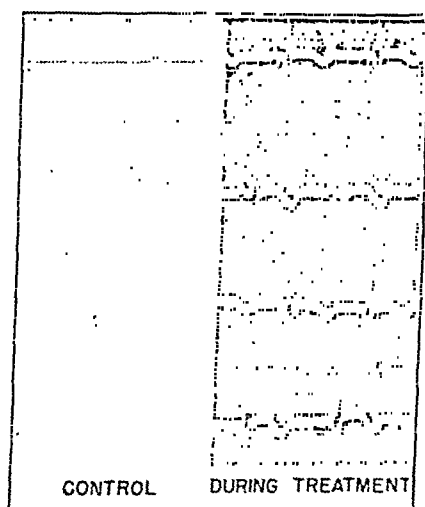


FIG. 3.—Electrocardiographic changes during administration of tartar emetic. "Control" record taken prior to treatment. "During Treatment" record taken 48 hours after last injection of 2S cc. 0.5% solution tartar emetic. Total dose 1.8 gm. (see text for schedule of administration). Note increase in height of P_2 and T wave inversion in all leads. Marked prolongation of QT interval (0.523; control 0.408).

T fusion was by necessity on an arbitrary basis. In normal records the most frequently occurring contour of the ST segment is a concavity upwards so that, as ST merges into an upright T wave, a change in direction of the curve to an upward convexity takes place.⁴ In many of our records the ST segment was convex at its origin and merged into a somewhat lowered T wave to form a single convex curve which could not be separated into ST segment and T wave components. To some extent this change is the natural result of decreasing the amplitude of the

some patients all leads were involved while in others, various combinations of the 4 leads showed involvement.

T Waves. It was in this component of the electrocardiogram that the most striking changes during antimony therapy occurred. These consisted of a decrease in amplitude of T waves in all leads, producing in a considerable number of patients diphasic or inverted T waves. Such changes were found to occur in 99% of the patients in this study. In order to classify the records as to degree of change caused by antimony, the actual

millimeter change in T-wave amplitude from the control records was measured to the nearest 0.5 mm. in Leads 1, 2, 3 and 4, and these were totalled for each patient. The range of this total change was from 0 to -21 mm.; 31 % of patients showed a total change from -0.5 to -5 mm., 41 % from -5.5 to -10 mm., 23 % from -10.5 to -15 mm., and 4 % from -15.5 to -21 mm. Typical T-wave changes are shown in Figures 1, 2 and 3.

Graybiel *et al.* in their excellent article⁴ have published percentage distribution curves on the amplitude of T waves in their 1000 young healthy aviators. Comparison of these with similar percentage distribution curves made on 100 control records taken on our patients before therapy reveal good correlation. The mean T-wave amplitudes of their 1000 cases were

study in Column 2. In Column 3 are the mean T-wave amplitudes of the records of our patients showing the maximum observed effect of antimony therapy, and in Column 4 the percentage change.

Figure 4 shows the frequency distribution curves in each lead before and during therapy. Solid lines represent control records and broken lines records taken during therapy. In each case the records chosen during therapy were those showing the maximum observed change but do not necessarily indicate the maximum change which occurred, since, as stated previously, the greatest antimony effect was frequently missed because of improper timing of the electrocardiogram during the course of treatment. Decreases in amplitude of the T waves are shown by a shift of the broken line curve to the left, *i. e.*, to the lower position on the

TABLE 1.—COMPARISON OF MEAN T-WAVE AMPLITUDE IN 1000 NORMAL SUBJECTS AND 100 PATIENTS BEFORE AND DURING ANTIMONY THERAPY

		100 patients			Decrease (%)
		1000 normal subjects (4)	Before antimony	During	
Lead 1	. . .	+2.55 mm.	+2 26 mm.	+0 88 mm.	59.4
Lead 2	. . .	+3 32 mm.	+3 17 mm.	+0 80 mm.	74 8
Lead 3	. . .	+1 33 mm.	+1 03 mm.	+0 04 mm.	103 9
Lead 4	. . .	+1 33 mm.	+4 93 mm.	+2 36 mm.	52 2

Lead 1 +2.55 mm., Lead 2 +3.32 mm. and Lead 3 +1.33 mm., as compared with our mean amplitudes of +2.26 mm. in Lead 1, +3.17 mm. in Lead 2 and +1.03 mm. in Lead 3. This favorable comparison indicates that the patients in this study comprise a fairly representative group of normals and establishes the fact that infection of these patients with schistosomiasis has, in itself, no effect on their electrocardiograms. Further, the fact that the mean amplitude of the T waves in our control records is within the range of normal indicates that antimony treatment which all patients had received from 1 to 3 months previously had no lasting effect on the T wave.

Table 1 shows a comparison of the mean amplitude of T waves of the 1000 cases analyzed by Graybiel *et al.* in Column 1 and of the 100 control records in this

scale, thus forming a definitely abnormal frequency distribution curve for records during therapy.

Changes in T-wave contour other than flattening of the peaks occurred in 24 % of the cases in this study. These changes consisted of notching of the T waves and occurred most frequently in Lead 4, occasionally in Leads 1 and 2 and rarely in Lead 3. Observation of the development and progression of this abnormality in serial records taken on the same patient during treatment affords an interesting clue as to the method of T-wave inversion in Lead 4 due to antimony. It was frequently observed in this lead that initially a notch appeared late in the T deflection, which in subsequent records became deeper, eventually descending below the iso-electric line to produce a + diphasic T₁ and finally with the complete disap-

pearance of positive deflection to form a deeply inverted T_4 . This apparent sequence of changes was never observed in the limb leads, even though some records showed deeply inverted T waves in all leads.

electrocardiographic changes persisted, a total of 109 records taken from 1 to 86 days after treatment were analyzed. From this analysis it was found that there existed such a wide variation in the persistence of changes that no definite con-

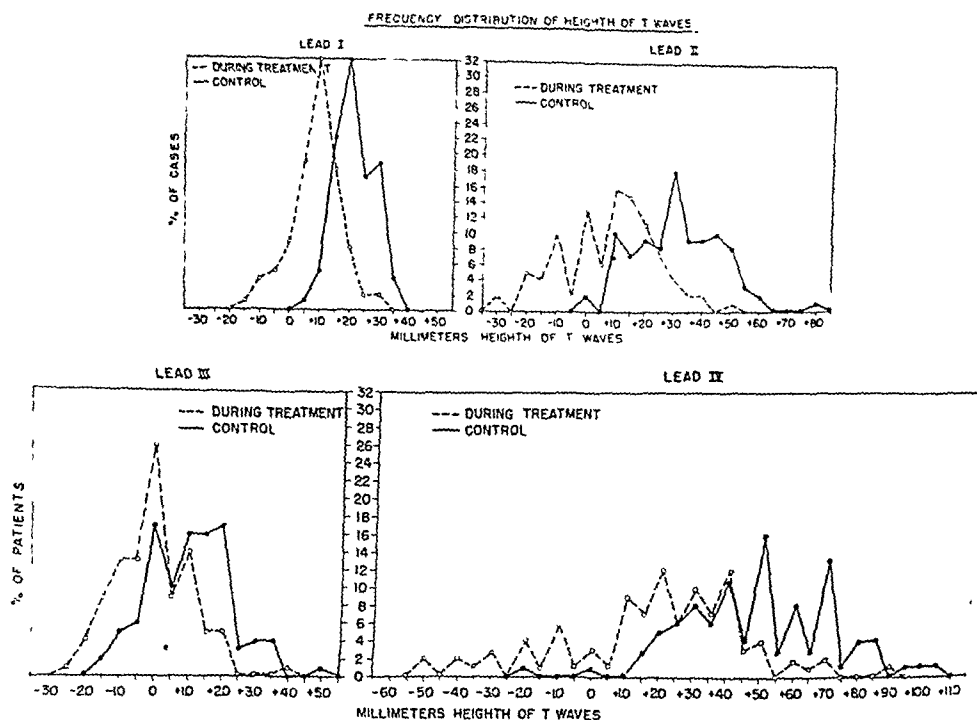


FIG. 4.—Frequency distribution in height of T waves in electrocardiograms taken before and during treatment with antimony in 100 patients. Note shift of treatment curve to left indicating frequency of decrease in amplitude or inversion of T waves in all leads during the administration of antimony (For discussion see text.)

NOTE.—One patient had a control record with an inverted T_4 . He had completed 40 cc. of fusidin 60 days prior to the taking of this record. His electrocardiogram was otherwise within normal limits.

On a few patients, frequent electrocardiograms were taken before, during and after injections in various periods during therapy. From these studies it was apparent that T-wave changes occurred gradually and progressively throughout the course of therapy. The earliest changes appeared after the first, second or third injection, and reached the maximum at or near the end of treatment. There was no tendency towards fluctuation from day to day nor was any regression towards normal observed during the 48 hour period between injections.

In attempting to determine how long after cessation of antimony therapy the

clusions could be drawn. Thirteen records were taken on different patients from 37 to 49 days after therapy and of these 6 had T waves equal in height to control levels, an additional 4 showed less than 30% of the original maximum change still remaining, while in 3 records 50 to 75% of the total observed change still persisted. Between 52 and 67 days after therapy complete return to normal was observed in 9 out of 15 records. Less than 25% of the total change still persisted in 4 records, and in the remaining 2 records 50 and 56% of the original total T-wave change was still present. In approximately 10% of these post-treatment

records the T waves returned to levels exceeding those of the control record. This curious change was noted in electrocardiograms taken on different patients from 18 to 78 days after cessation of treatment. No correlation was found between persistence of T-wave changes and the type or dosage of antimony treatment given. No attempt will be made to explain these results, other than to say that the major factor is apparently an individual variation about which we know little.

devised to correct for this variation. In this study "corrected Q-T" was calculated by the formula $Q-T = K\sqrt{RR}$ as advocated by Cheer and Dieuaide² in 1931. The mean "corrected Q-T" of all control records was 0.396 as compared with a mean of 0.422 for those records showing maximum antimony effect.

In addition, Q-T was plotted graphically against the cycle length, RR, as shown in Figure 5. In this graph the parallel lines representing the range of normal are those used by Feil³ in his

TABLE 2.—COMPARISON OF CHANGES IN AMPLITUDE OF T WAVES DURING TREATMENT WITH VARIOUS AMOUNTS OF FUADIN AND TARTAR EMETIC

Total change (mm.)	T.E. 1.45 gm (41 patients)	T.E. 1.8 gm. (21 patients)	Fuadin 70 cc (31 patients)	Fuadin 100 cc (7 patients)
0 to - 5	24 4%*	19 0%	42 0%	71 4%
- 5 5 to -10	51 2%	38 1%	35 5%	14 3%
-10 5 to -15	17 0%	38 1%	22 5%	14 3%
-15 5 to -21	7 4%	4 8%	0	0
Mean total change	-8 4 mm.	-8 8 mm.	-6 9 mm	-4 6 mm

* % in all instances represents % of total patients treated in each schedule.

Early in the course of this study, certain impressions were reached concerning the comparative effects of tartar emetic and fuadin on the electrocardiogram. Table 2 shows a classification of patients on different treatment schedules with regard to degree of total change produced by this treatment on the T waves in all leads. It must be remembered that these data are limited by the fact that the maximum change was not recorded in every case. In spite of this limitation it is obvious that tartar emetic has a greater effect on the electrocardiogram than does fuadin and that in the case of the former the larger the total as well as the daily dosage the greater the T-wave change. The disproportion between electrocardiographic effect and dosage of fuadin is due to (a) the limitation mentioned above and (b) to the fact that in this study adequate records were available in only 7 patients treated with 100 cc. of fuadin.

ELECTRICAL SYSTOLE. Variation of the Q-T interval with changes in heart rate has long been recognized by many investigators, and several formulae have been

paper on electrocardiograms and cardiac systole in pellagra. Control values are represented by solid circles and values during antimony therapy by open circles. Abnormally prolonged Q-T intervals occurred in 7% of the control records as compared with 27% of the records taken during treatment. Further analysis of the records showing prolongation of the Q-T interval reveals them to be proportionately distributed in relation to the treatment schedule used. This change is apparently caused with equal frequency by either fuadin or tartar emetic, and no significant difference was found between the different dosage schedules used in this study. In most cases, records with prolonged Q-T intervals also showed pronounced T-wave changes, although many patients with markedly lowered T waves had normal Q-T intervals. The mechanism of this increase in the duration of electrical systole is unknown.

PENTAVALENT ANTIMONY. On a few patients with leishmaniasis and filariasis, complete electrocardiographic studies were made during treatment with pentavalent

antimony compounds. Drugs used were neostibosan and stibanose. Patients on this class of antimony compounds showed similar changes to those seen with trivalent antimony, but to a much less marked degree. Indeed, some patients showed no changes whatsoever in electrocardiograms taken during treatment.

changes, but in 2 others showing equally marked T-wave changes, there was no slowing of the pulse. In only 2 instances did the heart rate of our patients fall below 60 beats per minute during treatment, 1 having a rate of 59 on the last day of therapy and the other a rate of 51 4 days after completion of therapy. In

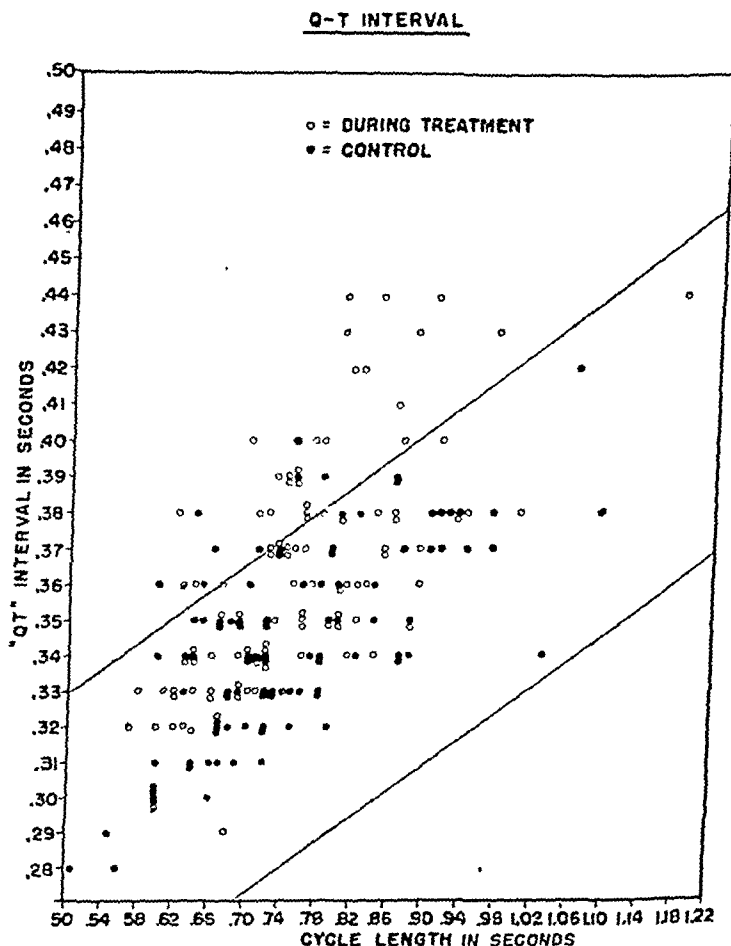


FIG. 5.—Correlation of QT interval and total length of electrical cycle in electrocardiograms taken before and during treatment with antimony in 100 patients. Note that 27% of patients during treatment exhibit prolongation in the QT interval. Normal range indicated by parallel lines (6).

Discussion. In this study the absence in the heart rate during antimony therapy is contrary to the findings of numerous other investigators, who frequently describe a pronounced bradycardia. Mainzer and Krause⁵ found a decrease in the heart rate in some of those cases showing the most marked electrocardiographic

many cases showing marked changes in other phases of the electrocardiogram there was actually an increase in the heart rate. In 2 patients, records taken before, during and after the actual injection of tartar emetic showed a slight increase in heart rate.

In our study, no attempts were made

to ascertain the underlying causes of these electrocardiographic changes. No lesions are found in the myocardium of dogs who die during the administration of large amounts of antimony.¹ The transient nature of the electrocardiographic changes with complete return to normal after varying periods is certainly presumptive evidence against the existence of actual myocardial damage. The fact that under close observation none of our patients developed any clinical signs or symptoms referable to the heart despite unlimited physical activity, except for 30 minutes bed-rest immediately following each injection, is additional evidence in this direction.

Mainzer and Krause⁵ discuss vagus stimulation as a possible explanation for antimony effect on the electrocardiogram principally because of bradycardia. However, those authors are not impressed by this explanation. The absence of significant change in the heart rate as found in this study also raises a valid objection to the autonomic theory, since it is well known that the rate is very sensitive to changes in autonomic balance. In addition, the persistence of electrocardiographic changes for as long as 60 days after the cessation of treatment and the absence of other signs of antimony effect upon the nervous system, both, cast considerable doubt upon an autonomic etiology.

Winkler *et al.*⁸ have studied the effect of increased potassium ion concentration upon the electrocardiogram in dogs. They consistently found an increase in T-wave amplitude and lowering and eventual disappearance of P waves, as the serum potassium concentrations rose. Since these changes are just the opposite from those observed in this study, it seems logical to conclude that the potassium ion present in tartar emetic is not an

etiologic factor in the electrocardiographic changes described in this paper.

Since the underlying mechanism producing these changes is at present unknown, their significance cannot definitely be established. However, it is our opinion that this is a relatively harmless side action of antimony, transient in nature, and of no great import as to impairment of cardiac function or as to prognosis of the patient. With the expected increase in use of antimony compounds in the next few years, it is of the utmost importance that these changes be recognized and their significance known, so that mistaken diagnoses of cardiac disease be avoided.

Summary and Conclusions. 1. An analysis was made of 315 electrocardiograms taken on 100 patients during various stages of treatment with tartar emetic and fuadin for schistosomiasis infection.

2. An increased amplitude of P waves in Leads 2 and 3 was found in 11 % of the patients.

3. A fusion of ST segment and T waves was found in 45 % of the patients.

4. Varying degrees of decrease in amplitude of T waves in all leads, resulting in deep inversion in many cases, were found in 99 % of the patients. This change was more pronounced during tartar emetic treatment than during fuadin treatment.

5. The Q-T interval was prolonged beyond the limits of normal in 27 % of the patients in this study.

6. The duration of these changes is variable, and has been noted up to 2 months after cessation of treatment.

7. The etiology and significance of these changes are unknown. It is our opinion that they represent a transient side action of antimony not indicative of cardiac damage or serious impairment of cardiac function.

8. Recent antimony therapy must be considered in evaluating abnormal electrocardiograms found in veterans and others.

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THROMBOSIS AND EMBOLISM OF PULMONARY VESSELS WITH SPECIAL REFERENCE TO PULMONARY VEIN THROMBOSIS

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RECENT reports on the use of such anti-coagulants as dicumarol and heparin in the various thrombotic and embolic states and the recent advances in the diagnosis, localization and treatment of phlebotrombosis in the lower extremities have awakened tremendous interest in the entire subject of thrombosis and embolism. For this reason a survey was undertaken based on the postmortem material observed at Bellevue Hospital. The purpose is to present the incidence of pulmonary emboli, thrombi and infarcts, their most frequent sources, the usual underlying disease entities that are present and the nature and evolution of the infarcts themselves. Such related questions as pulmonary venous thrombosis as a source of both bland and septic infarcts and the incidence of pulmonary systemic emboli in myocardial infarction were also studied.

In a series of 1000 consecutive post-mortem examinations on patients over the age of 20, excluding cases of subacute and acute bacterial endocarditis and deaths due to trauma, 109 (10.9%) were found to have pulmonary thrombi or emboli. Of these 109 cases, 84 (8.4% of total amount) had infarcts of the lung (Table 1). This latter figure is in agreement with the incidence generally reported. The majority of these patients were over the age of 50 with the peak incidence at the age of 60. There were no significant sex differences. It should be mentioned that very few surgical cases (postoperative) are represented in this series.

Two hundred consecutive necropsied cases in which pulmonary emboli were found (some not in original series) were

analyzed to determine the source of these emboli. Of these, 61 had thrombosis in the systemic veins, mainly the femoral and saphenous. Other venous sites were pelvic, lumbar, axillary, jugular and inferior vena cava. Mural thrombi in the right side of the heart were revealed in 55 cases (48 in the right auricle, 7 in the right ventricle). In the remaining 84 cases the site of the thrombosis was undetermined but was most likely located in the deep veins of the lower extremities, since none was found in the heart and permission was not granted for adequate dissection of the leg veins. It is also possible that in some of these undetermined cases, the vascular obstruction resulted from thrombi within the pulmonary arterial tree rather than from emboli. This may have been so especially in those cases with rheumatic heart disease. If these latter 84 cases are considered together with the known 64 cases containing systemic venous thrombi, there results a combined total of 148 cases in which the source for the emboli was extracardiac as compared to 55 cases in which the source of the emboli probably originated from within the heart (Table 2).

In a study undertaken by Hunter *et al.*¹ in which the leg veins were dissected in 350 consecutive necropsies, it was revealed that 52.7% had thrombi in the leg veins. In this series there were no significant sex differences; the average age was 59.78 as compared in 64.9 in those cases that did not have thrombi. This is an insignificant difference. In those cases having leg vein thrombi, 51 had pulmonary emboli; of these latter cases death was apparently due to pulmonary emboli

in 11 instances. The high frequency of thrombosis of the veins of the lower extremities is not generally recognized, particularly in view of the fact that in the great majority of the cases the first clinical evidence of their presence are manifestations of an embolus in the lungs. Hunter's⁴ figures are all the more significant because they are minimum figures, for in many of their cases it was impossible for them to perform as adequate a dissection of the lower extremities as they desired.

of systemic emboli in 48.7% cases. The incidence of embolic manifestations in these cases was about 3 times as great as in those cases in which mural thrombi were not present in the heart. One hundred consecutive necropsies (Bellevue Hospital series) of cases with pulmonary infarcts were compared with a series of 50 necropsied cases in which pulmonary emboli were present without infarcts (Tables 3 and 4). In the 100 cases with pulmonary infarcts it was found that 82 had heart disease, in 16 no heart disease

TABLE 1.—INCIDENCE OF PULMONARY ARTERIAL EMBOLI AND INFARCTS

Number of cases	1000
Number of infarcts	84 (8.4%)
Number with emboli without infarcts	25 (2.5%)
Total	109 (10.9%)

TABLE 2.—SOURCE OF EMBOLI IN PULMONARY ARTERIAL EMBOLIZATION AND INFARCTION

Number of cases	200
Systemic veins	61
Heart—Right auricle	48
Right ventricle	7
Undetermined	84

Thrombi in the axillary veins are an infrequent source of emboli in the lung. According to the review by Veal,¹⁰ in 1935, only 150 cases of axillary vein thrombosis had been reported during the preceding 50 years. However, in recent years many additional reports have appeared. Veal¹⁰ classifies thrombosis of the axillary veins under 2 groupings—primary, caused by trauma or bacterial infection, and secondary, usually due to malignant tumors of the chest region. Although axillary vein thrombosis is present it is an uncommon source of pulmonary emboli.

The frequency of intercardiac mural thrombi, especially in cardiac patients, as a source of both pulmonary and systemic emboli was well illustrated in a report by Garbin.³ In a study of 771 consecutive necropsies on patients with heart disease, 161 had mural thrombi in the chambers of the right side of the heart. Of these, 57.8% were associated with pulmonary infarcts; 193 cases had thrombi in the left side of the heart, that were the source

was present, the patients having died of a variety of diseases, such as carcinoma, tuberculosis, etc. However, the situation was quite the reverse in those cases having pulmonary emboli without infarction of the lung. In 50 such cases, 34 had no evidence of heart disease. This merely confirms the well-known fact that the combination of chronic passive congestion and obstruction of the pulmonary arteries is usually necessary before infarction will take place. However, this is not always necessary, as we have seen pulmonary emboli produce infarcts in the lungs in patients who do not have heart disease or any evidence of circulatory failure and who were not confined to bed.

Further study of the 100 cases with pulmonary infarcts with reference to the existing disease revealed that arteriosclerotic heart disease with or without hypertension was present in 34 cases. In these 34 cases, the source of the emboli was in the right auricle 10 times, the leg veins 10 times, and undetermined 14 times; 21 cases had arteriosclerotic heart disease

with either recent or old myocardial infarcts; of these the source of emboli in the lungs was the right ventricle in 3 instances, right auricle in 7, leg veins in 3, and undetermined in 8 cases. Of 20 patients with rheumatic heart disease, the source of emboli was in the auricle 11 times, leg veins once, and undetermined in 8 cases. Syphilitic heart disease with valvular involvement was present 5 times; periarteritis nodosa was present once; 18 patients did not have any cardiac disease (Table 5).

that serosanguineous, pleural fluid was present in 13 of 100 instances of pulmonary infarction. Serous fluid was present 34 times and no fluid at all in the remainder. When the pleural fluid could be with certainty attributed to the inflammation of the pleura resulting from the infarct, it was invariably found to be blood-tinged. In all of these instances the fluid was usually scant in amount and rarely exceeded several hundred cubic centimeters. In each instance when serous fluid was present, some degree of

TABLE 3.—UNDERLYING DISEASE IN 100 CASES OF PULMONARY ARTERIAL EMBOLI WITH INFARCTS

Arteriosclerotic and hypertensive heart disease	34
Arteriosclerotic and hypertensive heart disease with myocardial infarcts	21
Rheumatic heart disease	20
Syphilitic heart disease	5
Periarteritis nodosa	1
Non-cardiac disease	18

TABLE 4.—UNDERLYING DISEASE IN 50 CASES OF PULMONARY ARTERIAL EMBOLI WITHOUT INFARCTION

Non-cardiac disease	34
Cardiac disease	16

TABLE 5.—SOURCE OF PULMONARY ARTERIAL EMBOLI IN VARIOUS CARDIAC CONDITIONS

Type.	Auricle.	Ventricle.	Systemic veins.	Undetermined.
Arteriosclerotic and hypertensive heart disease	10	0	10	14
Arteriosclerotic and hypertensive heart disease with myocardial infarct	7	3	3	8
Rheumatic heart disease	11	0	1	8

It may be noted that thrombi in systemic veins as a source of pulmonary emboli are more frequent in arteriosclerotic heart disease than in rheumatic heart disease. This may be accounted for partially by the fact that arteriosclerotic heart disease occurs in the older age group and also that in some instances pulmonary infarcts and rheumatic heart disease may result from local pulmonary thrombosis rather than embolization.

The question of the clinical diagnosis of pulmonary infarcts at times presents great difficulties. For this reason the location, frequency and the fate of the infarcts, as well as the character of the pleural fluid associated with them was studied to determine whether these anatomic findings may be of aid in the clinical differential diagnosis. It was found

heart failure, edema and very often ascites existed. Therefore, the presence of this serous fluid could not be directly attributed to the effect of the pulmonary infarct on the pleura. As to the location of the pulmonary infarcts, it was found that they occur twice as frequently in the lower lobes as in the upper lobes. It was also noted that multiple infarcts are twice as frequent as a single infarct. There were no significant sex differences.

The fate of a pulmonary infarct was carefully studied by Castleman.² He combined roentgenographic studies with anatomic observations and found that infarcts gradually become replaced by fibrous tissue and shrink down to such an extent that they end as a fine, almost unobservable, fibrous scar. Very few of the pulmonary infarcts undergo liquefac-

tion necrosis. In our experience when this does occur, it is because they have resulted from an infected embolus or else have become secondarily infected. This is not a frequent occurrence. In the 100 consecutive cases in this series in which the actual number of infarcts numbered many more than 100, only 5 instances of liquefaction necrosis were noted. In 1 of these cases the source of the embolus was a thrombophlebitis of the femoral vein rather than the usual phlebothrombosis. In the other 4, the infarct occurred in a

cardial infarction. For a complete reference to the incidence of thrombotic and embolic phenomena in myocardial infarction, the reader is referred to the article mentioned above. These authors attributed the high incidence of embolic and thrombotic episodes present in cases of myocardial infarction to an increase of the clotting tendency of the blood based upon and associated with an accelerated plasma prothrombin clotting time.

Of the 100 cases of myocardial infarction, 48 had healed infarcts, while in 52

TABLE 6.—INCIDENCE OF INTRACARDIAC VENTRICULAR THROMBI IN 100 CASES OF MYOCARDIAL INFARCTION

Total cases of myocardial infarction:	100	Total cases of mural thrombi:	48
Recent infarcts	52		38
Healed infarcts	48		10
Anterior wall infarcts	79		43
Posterior wall infarcts	21		5
Location of mural thrombi:			
Left ventricle			38
Right ventricle			3
Both ventricles			7
			<hr/>
Total			48

TABLE 7.—INCIDENCE OF AURICULAR THROMBI IN MYOCARDIAL INFARCTION

Total cases	100
Right auricle	12
Left auricle	7
Both auricles	2
	<hr/>
Total	21

TABLE 8.—INCIDENCE OF EMBOLI IN MYOCARDIAL INFARCTION

Total cases	100
Pulmonary emboli	36
Recent infarct	23
Healed infarct	13
Systemic emboli	25

previously infected lung. It may be mentioned that many of the cases reported in the literature as isolated septic infarcts and, in particular, those associated with infections of the mouth and pharynx, may have been more properly classified as aspiration pneumonias, if adequate anatomic studies had been performed. However, several good examples of septic infarcts of the lungs have recently been reported by Hussey and Katz.⁵

In view of the recent report by Peters *et al.*,⁶ of the use of dicumarol in acute coronary thrombosis, we have reviewed 100 consecutive necropsied cases of myo-

infarcts were recent and unhealed. Some of the cases of recent infarction revealed slight areas of healed infarction. Of the 48 cases with healed infarcts, only 10 had mural thrombi in ventricles, while in 52 cases with recent infarcts 38 had ventricular thrombi. In other words the more recent the infarct, the greater the incidence of mural thrombi and hence, embolic phenomena; and as a corollary, the older the infarct, the lower the incidence of mural thrombi. Of the 48 cases with mural thrombi, the thrombus was located in the left ventricle in 38 instances, 3 in the right ventricle and in 7 thrombi

were found in both ventricles. Of these 100 cases, 21 had infarcts of the posterior wall and in 5 of these they were mural thrombi. Ventricular mural thrombi were found in 43 cases of anterior ventricular wall infarctions. The incidence of mural thrombi with anterior wall infarction is, therefore, much greater than in those cases with posterior wall infarcts (Table 6). In 21 of the 100 cases of myocardial infarction, thrombi were present as well in the auricles (right, 12; left, 7; both, 2) and many times were associated with ventricular thrombi (Table 7).

Emboli were found in the lungs in 36 of the 100 cases (Table 8).

The source of these emboli was a thrombus in the right auricle 14 times, the right ventricle 10 times, the leg veins 10 times and 2 undetermined. In 25 instances there were emboli from a thrombus in the left side of the heart, in the spleen, kidney, intestine or lower extremities. Frequently systemic and pulmonary arterial emboli were found in the same case. Emboli in the legs were present twice as frequently in those cases with recent myocardial infarction as in those with old infarction, probably indicating that these emboli contributed considerably to the death of the individual.

It was also observed in a routine study of cases having infarcts in organs other than the heart or lungs that at times one of the organs supplied by the systemic arterial circuit would reveal an infarct without any obvious source for an embolus, either in the heart, aorta or artery leading directly to the organ and the infarcted area. The only other probable source could therefore be from a thrombus in one of the pulmonary veins. With this in mind the pulmonary veins were carefully dissected in such cases. Within a short period, 15 cases were encountered having infarcts of the spleen, kidney or in both of these organs in which the source of the emboli was a thrombus in a pulmonary vein. Of these 15, 12 had extensive pulmonary tuberculosis, 1 had bronchiectasis, 1 had bronchogenic carcinoma and 1 had a pulmonary infarct.

Tuberculosis as a cause of pulmonary phlebitis is well known. That it occurs to such an extent that it results in a thrombus giving rise to systemic emboli, has infrequently been reported. Such an instance has been reported by Neel and Herrmann.⁷ In their case tuberculous pulmonary thrombophlebitis was associated with tuberculous peripheral arteritis. The arteritis in this case resulted from emboli originating from veins in the lungs. Medlar⁶ reported a case of coronary embolization from a tuberculous focus in a pulmonary vein. In this instance emboli contained tubercle bacilli and the resultant infarcts were infected with these organisms. However, in the instances recorded in this report, the thrombi were not infected and resulted in simple infarcts. The usual anatomic explanation is that tuberculosis in the parenchyma of the lungs involves the intima of the vein with the superimposition of the thrombus. It is interesting to note that the sites of pulmonary venous thrombosis were usually away from the areas of most extensive tuberculous damage. Other causes of pulmonary venous thrombosis have been listed by Brenner¹ as pneumonia, primary pulmonary vein sclerosis and pneumoconiosis. He does not mention pulmonary infarction as a cause of pulmonary venous thrombosis. In the 1 case of pulmonary infarction presented in this report, it was noted that the partially organized thrombi were present in the veins immediately surrounding the organizing pulmonary infarct. In this same case there were systemic arterial emboli and splenic and renal infarcts. Careful search revealed that the pulmonary veins could have been the only possible source for the emboli. This mechanism may account for some of those cases in which the embolization is considered clinically to be paradoxical. In the above-cited case an embolus from the femoral vein produced an infarct in the lung which in turn resulted in thrombus formation in an adjacent vein. This latter thrombus was in turn the source of emboli that were carried into a systemic arterial circuit. The usual explanation

given for those circumstances (paradoxical embolus), whereby emboli are said to pass through a patent foramen ovale under normal conditions, is not physiologically sound. The normal pressure in the left auricle is greater than that of the right; consequently an embolus would not pass from the right to the left auricle. However, under unusual circumstances the presence of a patent foramen ovale might account for a so-called paradoxical embolus. Such instances of paradoxical embolus can occur when a patient has first had preceding emboli in the pulmonary arteries resulting in pulmonary hypertension. The pressure in the right auricle has, therefore, become increased and any subsequent embolus would then be able to pass through a patent foramen ovale from the right auricle to the left auricle and give rise to systemic embolization.

Pulmonary venous thrombi are not as frequent as those in the systemic veins because the lung is in constant motion, it is protected from trauma by the bony chest cage and the veins are on the same general level with the heart, therefore, not necessitating the support of a long column of blood. Nevertheless, if more careful search were routinely made, undoubtedly more examples of pulmonary venous thrombi would be found.

Although not concerned with the main subject of this report, it was noted that in over 5000 postmortem examinations, pulmonary thrombophlebitis as a source for systemic emboli occurred in only 1 instance. In a review of the recent litera-

ture, we were only able to find reference to 1 similar case (Melbourne⁹). A pulmonary thrombophlebitis in the case noted in our series was secondary to a large lung abscess. Many infected systemic emboli were present. It is not obvious why the lung that is so frequently the site of pyogenic infections should infrequently be the source of infected emboli.

Conclusions. The general incidence of pulmonary emboli or thrombi in a series of 1000 consecutive necropsies (not inclusive of bacterial endocarditis and traumatic cases) was 10.9%.

The source of emboli in the lung is approximately twice as frequent from the systemic veins as from thrombi within the heart.

Pulmonary emboli in patients without heart disease frequently do not cause pulmonary infarcts.

In 100 consecutive necropsied cases of myocardial infarction, pulmonary emboli were noted 36 times and systemic arterial emboli 25 times. The incidence of pulmonary emboli is much greater in association with recent myocardial infarcts than with healed infarcts. The pulmonary emboli very often contributed to the death of the individual.

Pulmonary venous thrombi as a source of systemic arterial emboli occur more frequently than is generally recognized. Pulmonary tuberculosis was the disease most often associated with pulmonary venous thrombi.

Infected emboli resulting from pulmonary thrombophlebitis are exceedingly rare.

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RHEUMATOID ARTHRITIS

I. INTRODUCTION TO A STUDY OF ITS PATHOGENESIS*

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THE medical imagination has long been hypnotized by the notion that infection is in some way responsible for rheumatoid (atrophic) arthritis. This impression has largely been based upon confusion with the type of arthritis which is metastatic from a focus of infection, upon superficial resemblances between rheumatoid arthritis and infectious processes and upon certain serologic observations.

TYPICAL RHEUMATOID ARTHRITIS. It cannot be too strongly emphasized that the papers which follow are applicable only to *true* rheumatoid arthritis, a disease state which has been described by many authorities as a recognizable syndrome, if not an entity. In order that there be no misconception in the mind of the reader, its most characteristic clinical features are recapitulated as follows: a gradual onset between the ages of 20 and 40; a predilection for thin females living in a temperate climate; a fairly symmetrical involvement of the extremities, particularly knees, wrists and proximal interphalangeal joints of fingers by an indolent, painful, tender, somewhat doughy, fusiform swelling with more of local heat than redness; and the gradual development of deforming contractures through a series of remissions and exacerbations until the advent of bony or fibrous ankylosis brings the pain to a close. It should be axiomatic that (hypertrophic) osteoarthritis deviates from this clinical pattern in so many respects (to say nothing of roentgenographic and gross pathologic differences) that its status as a separate disease is clear. Other types of arthritis which for the sake of clarity

will be omitted from present consideration include Still's disease, the ankylosing spondylitis of Marie-Strümpell, Felty's syndrome, "menopausal" arthritis, "psoriatic" arthritis, and arthritis secondary to a focus of infection.

In selecting cases of rheumatoid arthritis for serologic study we have used the following criteria: disease duration of at least a year and a fairly symmetrical involvement of proximal interphalangeal joints of fingers. The "activity" of the disease process was disregarded.

ARTHRITIS FROM FOCAL INFECTION. In synovitis attributable to a focus of infection, it is assumed that bacterial toxins and not the bacteria themselves are carried to the joint in the blood stream. Such metastatic toxins probably have no "arthrotropic" intentions. It is rather that the joints respond selectively to the presence of the toxins in the general circulation because, as large tissue spaces¹ endowed with an abundant blood and nerve supply, they are physiologic sounding boards. It is for this reason that the synovial reaction is a dominant feature of such systemic disturbances as the antigen-antibody interactions occurring in rheumatic fever during the acute phase (*cf.* Coburn and Pauli⁶) or in serum disease. Once a synovial reaction to a substance arriving *via* the blood stream has become established it tends to become more intense because a local increase in capillary permeability permits the deposition of still larger amounts of the causative agent (*cf.* Menkin¹⁴). Arthritis due to metastatic toxin will thus be no more symmetrical

* The series of papers of which this is the first has been supported by grants from the Charlotte Drake Cardeza Foundation of the Jefferson Medical College and the National Foundation for Infantile Paralysis, Inc.

than are the joint lesions of rheumatic fever, and its only characteristic feature will be its response to alterations in the foci of infection. Typical examples observed for a sufficient time present a clear distinction between rheumatoid arthritis and arthritis which is metastatic from a focus of infection. The latter is restored toward normal when the focus subsides, and the former is either unaccompanied by demonstrable foci or uninfluenced by their treatment. Atypical cases, of course, are confusing and it is possible that both types may coexist in the same patient or even in the same joint. The present discussion is concerned only with those cases of rheumatoid arthritis which approximate the syndrome described above as "typical."

An open-minded approach to the baffling problem of the pathogenesis of rheumatoid arthritis must be unhampered by the traditional concept of a bacterial etiology. Although resting on insecure evidence, this concept has persisted strongly and has been the greatest single obstruction to a solution of the problem.

THE THEORY OF BACTERIAL ETIOLOGY. A brief inspection of the foundation of the bacterial theory will help to place it in its proper perspective. It had its origin in an era of enthusiasm for assigning infectious causes to diseases. It was fostered by the presence of external signs of inflammation accompanied by an increased rate of erythrocyte sedimentation, and it was strengthened by observation of patients in whom a frank focus of infection was undeniably related to arthritis. It was subsequently buttressed when a similar arthritis was produced in experimental animals by bacterial injections and in many minds it was conclusively established by the demonstration of strong agglutinins for the hemolytic streptococcus in the serum of many patients with typical rheumatoid arthritis.

These various lines of evidence, however, will not bear close scrutiny. Inflammation does not necessarily signify infection, as the signs and symptoms in gout

or fresh burns will bear witness; nor does a rapid sedimentation rate necessarily signify infection, as observations¹⁰ of an increased rate in gout will testify. The fact that focal infection can cause arthritis does not mean that all non-specific arthritides have the same cause. The experimental argument loses weight when we consider that turpentine injections are capable of arousing an arthritis which grossly and microscopically closely resembles that produced by injections of bacteria.⁵ The significance of the streptococcus agglutinins will be discussed in a subsequent paper.

ARGUMENTS AGAINST A BACTERIAL ORIGIN. The presence of bacteria of any species has not been consistently demonstrable in the blood of patients with rheumatoid arthritis.¹² The extraordinary prolongation of the active stage of the joint lesions in this disease and their tendency to symmetry are arguments against bacterial etiology in general, and their indifference to sulfanilamide⁷ and penicillin^{3,8} is against a streptococcal etiology in particular.

**ANTISTREPTOLYSINS AND ANTIFIBRINO-
LYSINS.** Antibodies formed in response to the presence of the whole hemolytic streptococcus may be expected to be directed not only against the different fractions which comprise its complex structure but also against the toxins or products of the microorganism. Thus, known hemolytic streptococcal infections such as scarlet fever or erysipelas or presumed hemolytic streptococcal infections as rheumatic fever ordinarily arouse antibodies which oppose the action of streptococcal hemolysin (antistreptolysin) and streptococcal fibrinolysin (antifibrinolysin). In rheumatoid arthritis these antibodies have not been found in significant titer.

The first report on antistreptolysin in the serum of rheumatoid arthritis was that of Myers and Keefer¹⁵ who stated that it was not present in excess of normal. Dawson and Olmstead⁹ found no definite increase in antistreptolysin in cases of more than 1 year's duration but recorded

some increase in earlier cases. Slight increases in a few cases were reported by Blair and Hallman² and by McEwen, Bunim and Alexander,¹³ but the levels found were not comparable with those in rheumatic fever and known hemolytic streptococcal infections. Discrepancies in these observations may be accounted for by variations in selection of cases; different criteria for a normal level and disregard of antecedent upper respiratory tract infections. In 1940 Bunim and McEwen⁴ concluded that antistreptolysin is not increased in rheumatoid arthritis. With regard to antifibrinolysin, Myers, Keefer and Holmes,¹⁶ McEwen, Bunim and Alexander,¹³ and Perry¹⁷ are in agreement that

rheumatoid arthritis sera are within normal limits.

Reports of attempts to demonstrate complement-fixing antibodies and cutaneous reactions to streptococcus fractions in rheumatoid arthritis have been summarized by Hench¹¹ as contradictory and inconclusive.

Summary. The importance of the distinction between true rheumatoid (atrophic) arthritis and arthritis of other types, particularly that which is secondary to a focus of infection, is emphasized.

The foundations of the bacterial theory, and more specifically, the hemolytic streptococcal theory, of the origin of rheumatoid arthritis are examined and found insecure.

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RHEUMATOID ARTHRITIS

II. NON-SPECIFIC SEROLOGIC REACTIONS*

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In the course of a study of precipitins in the sera of patients with rheumatoid (atrophic) arthritis, to be reported in subsequent papers, peculiar non-specific properties were noted in the sera. These properties appear to exert an influence of primary importance on the general serology of this disease.

PRECIPITATION WITH PHYSIOLOGIC SALT SOLUTION. The "negative" or "saline control" tube of each precipitin test contained 0.4 cc. of 0.85 % sodium chloride solution, 0.1 cc. of serum and no antigen. Each test was incubated in a water bath at 37° C. for 2 hours and refrigerated overnight before being examined. The sera of many patients with typical⁶ rheumatoid arthritis of more than 1 year's duration have a tendency to precipitate in this "negative" control tube. Before centrifugation, the precipitate may often be seen (with a reading glass, fluorescent lamp, dark background and darkened room), appearing as fine particles resembling those of a genuine antigen-antibody reaction. Like the latter, their intensity increases day by day as the period of observation is prolonged. With centrifugation, the tendency of arthritis serum to precipitate in saline becomes still more marked. The precipitate is now apt to take the form of flakes, visible with the unaided eye. These likewise resemble the flakes of an antigen-antibody reaction, except that the arthritis serum flakes are occasionally more translucent. Of the control sera, from patients with diseases other than rheumatoid arthritis, a few have shown fine particles in the saline control before centrifugation, visible only with the reading glass, but none have been encountered which yielded flakes on cen-

trifugation. The uncentrifuged non-specific precipitation of arthritis serum increases with the age of the serum used in setting up the test. Usually it is not well marked until the serum has stood in the refrigerator for about 1 week. It is not abolished by fasting the patient overnight before drawing blood. The centrifuged non-specific precipitate is less influenced by the age of the serum. Its intensity bears only a general, not a closely quantitative relation to that which occurs before centrifugation. With the passage of time there is also apt to be some spontaneous precipitation in the refrigerated stocks of undiluted serum, a possible source of error in precipitin testing. Precipitation in the saline controls of arthritis serum precipitin tests after centrifugation has been recorded by Dawson, Olmstead and Jost⁴ and by Chasis and McEwen,² but its importance does not seem to have been recognized.

Furthermore, experiments which will be reported in a subsequent paper suggest the disconcerting possibility that an antigen may become non-specifically enmeshed in the saline precipitate of rheumatoid arthritis serum during centrifugation, thereby increasing the intensity of the precipitate, *even though the serum contains no homologous antibodies*. It is obvious that the existence of this possibility would make it difficult ever to be certain that such a serum actually contained precipitins specific for *any* antigen. Less frequently, the reverse situation has been encountered, in which the presence of an antigen has obstructed the tendency to precipitate in saline. The enhancement of precipitation by an apparently heterologous antigen is best seen after centrifugation, but this effect may often

* Aided by grants from the Charlotte Drake Cardeza Foundation of the Jefferson Medical College and the National Foundation for Infantile Paralysis, Inc.

be detected in the delicate particles which are present before centrifuging. Omission of centrifugation will therefore not prevent its occurrence.

To overcome the tendency of arthritis serum to precipitate spontaneously with saline, we have tried the following measures, without success: substitution of 1.7 and 3.4% sodium chloride for 0.85 and swinging the undiluted serum in an angle centrifuge before setting up the test. Samples of serum were also diluted with 3 parts of physiologic salt solution, placed in a water bath at 37° C. for 2 hours, refrigerated for 7 days and cleared in an angle centrifuge. When these sera were used in a precipitin test, no precipitate occurred in the saline control tubes, but the potency of precipitins presumed to be originally present was greatly impaired.

AGGLUTINATION OF COLLODION PARTICLES. During an investigation of streptococcus precipitins in the serum of rheumatoid arthritis we undertook to convert a precipitin reaction into an agglutination by sensitizing a particulate vehicle with the antigen. Suspensions of fine collodion particles were prepared by the method of Cannon and Marshall¹ and it was found that rheumatoid arthritis sera possessing strong hemolytic streptococcus agglutinins were apt to agglutinate the particles even when they had not been sensitized by exposure to an antigen. The "collodion titers" of the arthritis sera varied rather widely with different batches of collodion particles. They ranged up to 1:2560 and could not be consistently correlated with the streptococcus agglutinin titers of the same sera. Most of the normal sera examined did not give this reaction at any

strength. None was positive beyond the lowest final serum dilution used, which was 1:20. The collodion-agglutinating factor in arthritis serum is rendered inert by exposure to 65° C. for 30 minutes. Ammonium sulfate fractionation shows that it resides in the serum globulin. The agglutination of collodion particles by rheumatoid arthritis serum is apparently a non-specific flocculation analogous to the Takata-Ara, colloidal gold, cephalin-cholesterol and formol gel reactions. It is not recommended as a diagnostic test because of wide variation in the reactivity of successive batches of particles and because it is sometimes consistently negative against sera from patients with well-established rheumatoid arthritis of many years duration. Its relation to the titer of hemolytic streptococcus agglutinins will be discussed in a subsequent paper.

The tendencies of rheumatoid arthritis serum to precipitate in saline and to agglutinate suspensions of collodion particles are probably interrelated. Both are also presumably related to the globulin content of this serum, which is known^{3,5} to be high.

Summary. The sera of many patients with well-established rheumatoid (atrophic) arthritis have a tendency to precipitate in the saline control tube of a precipitin test, especially after centrifugation. Many rheumatoid arthritis sera also differ from the normal in being able to agglutinate suspensions of fine collodion particles.

Conclusion. Supposedly specific reactions in which rheumatoid arthritis serum takes part should be interpreted with caution.

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RHEUMATOID ARTHRITIS

III. THE PNEUMOCOCCUS ANTIBODIES*

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As an adjunct to their study of hemolytic streptococcus agglutination by the sera of patients with rheumatoid (atrophic) arthritis, Dawson, Olmstead and Boots^{3,4} have investigated the agglutinability of other gram-positive cocci by the same sera. Agglutination to a significant titer did not occur with *Strep. viridans*, *Strep. anhemolyticus*, *Staph. albus* or encapsulated pneumococci of Types 1, 2 or 3. However, non-encapsulated pneumococci derived from any of these types were agglutinated almost as strongly as the hemolytic streptococci. It is the purpose of the present paper to provide an explanation for the existence of agglutinins for the non-encapsulated pneumococcus in the serum of rheumatoid arthritis.

Materials. Bacteria and Culture Media. The non-encapsulated pneumococcus strain I/192/R was used throughout. Stock cultures were kept in tubes in the refrigerator in a few cc. of neopeptone broth² (extract of 1 pound of top of the round, grossly fat-free; neopeptone [Difco], 10 gm.; sodium phosphate dibasic, 4 gm.; tap water to make 1000 cc.; final pH 7.6 to 7.8) with the addition of a few drops of defibrinated horse blood. The same medium was used in preparing suspensions for agglutination. Unless the stock was subcultured at least once a month it lost its agglutinability. Mrs. Miriam Olmstead Lipman kindly furnished samples of the microorganism on several occasions. Cultures for extractions were grown in a tryptone glucose yeast extract broth¹⁴ (tryptone peptone [Bacto], 20 gm.; glucose [cerelose], 5 gm.; NaCl, 5 gm.; yeast extract [Bacto], 2 gm.; dipotassium phosphate, 2.5 gm.; distilled water, 1000 cc.; final pH, 7.5). The pneumococci tended to

die out after 2 or 3 subcultures unless blood was again added.

*Pneumococcus C Substance.*¹⁶ For quantitative serologic use the polysaccharide was prepared by an approximation of the method of Goebel, Shedlovsky, Lavin and Adams,⁸ omitting the removal of protein with copper sulfate. The acetone-precipitated polysaccharide resisted drying by suction and was dried from the frozen state by Mr. Joseph Smolens, to whom our thanks are due. About 12 mg. of a fluffy white substance were obtained from 1 liter of broth culture. For qualitative serologic use a simpler method based on the hot formamide extraction procedure of Fuller⁶ yields a satisfactory product. Freshly sedimented organisms were resuspended in formamide (Eastman No. 565), 1 cc. per 100 cc. of original culture medium, and heated in a mineral oil bath at 150° to 155° C. for 15 minutes. This treatment rendered the fluid nearly transparent, indicating efficient bacterial disintegration. After cooling, the pH was adjusted to approximately 4.5, whereupon a light flocculent cloud appeared. This was spun down and discarded. It presumably included the F polysaccharide of Goebel, Shedlovsky, Lavin and Adams.⁸ This is the C substance conjugated with a fatty acid. If its removal was omitted a second peak appeared in precipitin tests, attributable either to the action of anti-C in a different optimal range or to anti-F (cf. Goebel and Adams⁷). To the supernatant were added sodium acetate (about 10 mg. per cc.) and 3 volumes of 95% ethyl alcohol. After overnight refrigeration the copious precipitate was collected in the centrifuge and taken up in physiologic salt solution, to 2% of the original broth volume. This extract was neutralized and a large undissolved residue was discarded by centrifugation. "Mer-

* Aided by grants from the Charlotte Drake Cardeza Foundation of the Jefferson Medical College and the National Foundation for Infantile Paralysis, Inc.

thiolate" (sodium ethyl mercuri thiosalicylate, Lilly) in final concentration 1:10,000 was added as preservative.

Rheumatoid Arthritis Sera. These were from patients with typical¹⁷ rheumatoid arthritis. They were obtained in the dispensaries and wards of the Hospital of the University of Pennsylvania, Temple University Hospital and the Philadelphia General Hospital. Preservative was added as in the case of the C substance. The tendency of such sera to precipitate spontaneously in the saline control tube of a precipitin test has been described elsewhere.¹⁸

Sera From Patients Without Rheumatoid Arthritis. These were obtained on the surgical and medical wards of the Hospital of the University of Pennsylvania. The patients had a wide variety of diagnoses; none complained of arthritis; all were afebrile. Their age range was comparable to that of the arthritics. Preservative was added as in the arthritis sera.

Methods. Precipitation Tests. The samples of arthritis serum were allowed to age in the refrigerator about 1 week, and the non-arthritis sera at least that long, before setting them up in precipitin tests. A few sera had enough opalescence to render the precipitates illegible. The tubes used were 10 x 75 mm. The antigen was used in amounts ranging downward from 0.4 cc., the volumes being made up to 0.4 cc. with physiologic salt solution, and to each tube was added 0.1 cc. of serum. With each test was included as negative control 0.4 cc. of saline without antigen. The tube contents were mixed by tapping and the tubes were incubated in a water bath at 37° C. for 2 hours. After overnight refrigeration readings were made with the aid of a reading glass (2 inch diameter, 4 inch focus, magnification 2X) held about 2 inches from the tube, a fluorescent lamp, a dark background and a darkened room. The tubes were tapped several times to make the precipitate rise and become evenly distributed. Too vigorous tapping was avoided as tending to break up the larger aggregates. Centrifugation of the tests was omitted because it aggravates the tendency of arthritis serum to precipitate in the control tube. Subsequent readings were made at daily intervals for 1 week, the tubes being refrigerated between readings. Aside from covering the tube rack with an unsterilized paper towel,

no special precautions were taken to secure sterility. Visible bacterial contamination was not encountered.

Agglutination Tests. The procedure of Goebel and Adams⁷ was used, with these exceptions: the bacterial suspension was diluted to approximate Tube 4 of the McFarland¹³ turbidity scale; the amounts of suspension and serum dilution used were both 0.3 cc. The last tube in which agglutination was visible with a reading glass was taken as the titer. This was usually 1 tube higher than the last one in which agglutination was visible with the unaided eye. Substitution of triple for single washing of the organism had no effect on the titer.

EXPERIMENTAL. Our concept evolved in the following manner: Arthritis sera possessing hemolytic streptococcus agglutinins in high titer were set up against suspensions of non-encapsulated pneumococci, which they agglutinated in titers as high as 1:640. A few sera from normal persons, included as controls, agglutinated the pneumococci in titers of zero, 1:20 or 1:40, values comparable to Group A hemolytic streptococcus agglutinin titers of patients without rheumatoid arthritis.³ As will appear later, these controls were not representative, but at the time we thought arthritis sera contained significant pneumococcus agglutinins. We then sought the pneumococcus component against which the arthritis antibodies were directed. The negative results of Dawson, Olmstead and Boots with encapsulated organisms indicated that this was a somatic fraction. An obvious candidate was the pneumococcus C substance,¹⁶ which indeed proved precipitable by the serum of rheumatoid arthritis.

At this point we learned of the observation of Heidelberger and Anderson⁹ (subsequently amplified¹⁰), that the sera of most normal persons contain measurable amounts of precipitin for the pneumococcus C substance. To determine if the precipitates with rheumatoid arthritis sera were explicable on this basis, sera from patients without rheumatoid arthritis were set up in the same manner. Com-

parison of the precipitation by sera from non-arthritic patients (Table 1, A) with that by sera from patients with rheumatoid arthritis (Table 1, B) shows that the results with the 2 kinds of serum are about the same, except that arthritis serum has a greater tendency to precipitate in the saline control and that its precipitation with the antigen is heavier when the saline control is positive. When allowance is made for this factor, arthritis serum has no more than the normal quota of anti-C.

these agglutinins would appear to be stronger in the serum of a person who had acquired rheumatoid arthritis. Agglutinin titers are included in Table 1. Those of arthritis sera average about 2 tubes higher than those of non-arthritis sera, confirming our suspicion that the pneumococcus antibodies in arthritis serum are actually within normal limits but that their action is enhanced by the presence of a non-specific property related to the arthritis.

TABLE 1.—PRECIPITATION OF PNEUMOCOCCUS C SUBSTANCE

A. By Representative Non-arthritis Sera							
Antigen gamma	Cha *160	Vec 160	McN 80	Hop 80	Mor 40	Kau 40	Sel 0
0.5	?fthhhh	oooo?vv	offh = = =	?? ?vvvv	oooovfff	oooooooo	o?vffft
0.2	ooooovvf	oooooooo	o?vt h t t	vvvfff t	ooooovvv	oooooooo	o?vvvff
0.05	oooooooo	oooooooo	oo? v v v	?vvvvvv	ooooo?v	oooooooo	oo?oo?v
0	oooooooo	oooooooo	oooo o o o	??oo?vv	oooooooo	oooooooo	oooooooo
B. By Representative Rheumatoid Arthritis Sera							
Antigen gamma	Les *640	Gre 320	Lak 160	Kep 80	Far 40	Bat 40	Bro 20
0.5	fth = - - -	fth = - - -	o?vt t t t	fh + + - - -	ovfttff	oooooooo	oooo??v
0.1	fh = + - - -	?vf h - - -	oooovff	vft h - - -	ovftttt	oooooooo	ooooovvf
0.05	ft = = - - -	?vf t - - -	oooovff	vvf f - - -	ovfffff	oooooooo	oooo?vf
0	vff t - - -	o?vv - - -	oooooooo	vvf f - - -	ovfffff	oooooooo	oooooo?

2×10^{-5} and 2×10^{-6} dilutions of antigen were used in setting up the tests. All antigen amounts were made up to 4 cc. with physiologic salt solution. Each tube received 0.1 cc. of serum. Incubation in water bath at 37° C. for 2 hours. The first reading was made after overnight refrigeration. Subsequent readings at daily intervals, the tubes being refrigerated between readings. They were not centrifuged.

The 7 columns of symbols recorded for each serum represent readings made on the first through 7th days after setting up the test. The following is the interpretation of the symbols:

Visible only with reading glass:

0 = negative

? = doubtful

v = very faint trace

f = faint trace

t = trace

h = heavy trace

Without reading glass:

= = weakest visible

+ = next stronger

- = reading not made

* The figure beneath the designation of each serum donor represents the titer of agglutinins in that serum for non-encapsulated pneumococcus. Thus, 160 signifies that a final serum dilution of 1:160 was the highest (of serial double dilutions) capable of causing agglutination.

The serum of patients with typical rheumatoid arthritis thus appears to have the ability to enhance serologic aggregations. The non-specific nature of this enhancement is emphasized by the observation¹⁵ that such sera can agglutinate suspensions of fine collodion particles which have not been sensitized by the addition of an antigen. The implication with regard to the agglutination of non-encapsulated pneumococci by rheumatoid arthritis serum is obvious: if most normal sera contain agglutinins for this organism,

Discussion. What portion, if any, of these agglutinins is actually anti-C is a question which has no bearing on our present argument. Presumably the anti-C in normal human serum is due to the frequent presence of pneumococcus in the normal throat. This makes it likely that normal sera, and hence arthritis sera, contain antibodies against somatic pneumococcus fractions other than C. Agglutinins directed against other fractions may account for the lack of a closer correlation

between agglutinin titers and strengths of C precipitins in Table 1.

The long period of observation of precipitates in these experiments is necessitated by the inherent feebleness of human anticarbohydrate antibodies in a precipitin reaction, as compared with those of some other species, notably the rabbit. Another example is the weakness of human antisera in precipitating homologous type-specific carbohydrates of pneumococcus, as Finland and Sutliff,⁵ Stats and Bulowa¹⁵ and others have pointed out. These species differences have been attributed by Horsfall and Goodner^{11,12} to qualitative differences in the phospholipid requirements of the respective antibodies when united with antigen. It is worthy of note that Heidelberger and Anderson⁹ allowed human anticarbohydrate precipitates to stand 8 days in the refrigerator before collecting them for quantitative analysis.

In recording our results, designations of various degrees of "trace" for precipitates visible only with a reading glass, in addition to the more conventional plusses for grosser precipitates, have been used for the sake of emphasizing several facts, namely, that these precipitates are more delicate than those obtained with rabbit antisera, that special aids (reading glass, fluorescent lamp, dark background, darkened room) are necessary for their evaluation, and that gradations observed under these circumstances are genuine. When dealing with slight gradations, in the absence of a permanent standard for comparison, it is to be expected that a given precipitate might not always receive the same rating. In practice, the range of such variation in reading is small. For example, a "trace" might on another

occasion be called a "faint trace" or a "heavy trace;" but this discrepancy does not exceed 1 gradation. The significant feature, however, is that on simultaneous comparison of a series of tubes, the relative intensities of the precipitates are easily determined. The reliability of the method is further demonstrated by examining a set of tubes on succeeding days, without knowledge of previous readings. The heaviest precipitate is found in the same tube as before, with comparable increases in all tubes.

The pneumococcus phase referred to herein as non-encapsulated is widely known as "rough." In the more logical later terminology of Dawson¹ it would be "smooth," but this classification has not received the sanction of usage.

Summary. With special visual aids and prolonged observation as substitutes for centrifugation, rheumatoid arthritis sera are found to precipitate the somatic C carbohydrate of pneumococcus a little more strongly than do sera from non-arthritis patients. The greater ability of arthritis sera in this regard is apparently related to their greater tendency to precipitate in the saline control tube of a precipitin test.

Rheumatoid arthritis sera likewise agglutinates non-encapsulated pneumococci a little more strongly than do sera from non-arthritis patients. This difference is presumably related to the ability of arthritis sera to agglutinate collodion particles.

Conclusion. The increased ability of the serum of patients with rheumatoid (atrophic) arthritis to agglutinate non-encapsulated pneumococci appears to be due to a non-specific enhancement of the action of normally present antibodies.

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PROGRESS OF MEDICAL SCIENCE

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UNDER THE CHARGE OF

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CHANGING CONCEPTS IN MEDITERRANEAN (COOLEY'S) ANEMIA

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TERMINOLOGY. In 1889, von Jaksch⁷⁹ described an anemia accompanied by splenomegaly and leucocytosis which he thought was of specific nature and to which he gave the name "Anæmia infantum pseudoleucæmica." This was subsequently called "Jaksch-Hayem-Luzet's"⁷¹ anemia after the names of the authors who described it more fully. Later writers included under the name of von Jaksch's anemia a miscellaneous group of unrelated anemias and splenomegalies.

There appeared in 1921, in the clinic of Thomas Cooley, a brother and sister with anemia, splenomegaly, and distinctive skull changes associated with a mongoloid appearance. Other similar patients were noted over the next few years. In 1925 the series was described to the American Pediatric Society by Cooley and Lee.^{22a} Not until 2 years later did Cooley and his associates^{21a,23} assert that his 7 cases plus 5 others reported by Stillman and Pool⁷⁰ belonged to a definite clinical entity, a hemolytic dysfunction distinct from hemolytic icterus and sickle cell anemia, to be differentiated from other kinds of cases of the von Jaksch's type. The concept of von Jaksch's anemia promptly began to fade from prominence.

Debré and his associates²⁸ claim that Hayem described the condition in 1898 calling the disease "splenomegalic icterus," and that Minkowski described it again in 1900. Perusal of Debré's report makes it evident, however, that no clear line was drawn between what are now known to be 2 distinct entities, namely, Mediterranean anemia and congenital hemolytic (spherocytic) jaundice. Most writers credit Cooley as the true discoverer of the disease, for it was he who first challenged the specificity of von Jaksch's anemia and reclaimed a genuine clinical entity from the confused mass of disorders grouped under that term.

Cooley proposed the name "erythroblastic anemia"^{21b} because of the large numbers of immature red cells in the peripheral blood. Later developments have shown that this term was not the best,⁴³ as Cooley himself later realized, for it causes confusion between this specific entity and other anemias characterized by erythroblastosis such as "erythroblastosis foetalis" due to the Rh factor. The term erythroblastic anemia has become further unsuitable with the uncovering of the "carrier" state and of mild clinical and subclinical forms in which

nucleated red blood cells may be absent. In truth, anemia itself is not an essential diagnostic criterion.

Because of the predilection of the disease for children of Greek, Italian, Syrian or Armenian parents, Whipple and Bradford^{83b} suggested "Thalassemia" or "Mediterranean anemia" from the Greek word for "great sea." Cooley, feeling the use of his own name for the designation of the disease added another undesirable eponym, adopted the term Mediterranean anemia for his own clinic.^{21d}

The severe and readily recognizable form originally described by Cooley has assured the anemia its place in the standard pediatric texts. However, more recent investigations^{68b,c,86} are showing that the disease has a wide range of clinical severity and that mild cases may have no anemia and normal life spans. Mediterranean anemia has thus spread from the bounds of pediatrics to invade the realm of adult medicine. This has further complicated terminology. Mediterranean "disease" would seem more accurate than "anemia." Smith^{68b} uses the terms "severe" and "mild" for the gradations of severity. Valentine and Neel^{75a} propose "thalassemia minor" and "thalassemia major" for the corresponding conditions. Under "target-oval cell syndrome" Damashek^{26c} includes the entire range of variation from the frank advanced case to the patient who presents naught but minimal hematologic abnormalities.

THE CARRIER STATE. Some years ago, Angelini⁴ and Caminopetros^{15a} proposed the existence of a genetic carrier, as evidenced by blood studies, results of the fragility test, and mild roentgenographic changes. Caffey^{14a} also had hypothesized that mild cases can reach adult life and transmit the disease.

Wintrobe's⁸⁵ confirmation of Angelini's and Caminopetros' observation that the parents of active cases had decreased fragility of the red blood cells supplied the final link. This related to Mediterranean anemia various other anemia syndromes such as Damashek's^{26a} "Fami-

lial Mediterranean Target-oval Cell Syndrome," Strauss and Daland's⁷² "Familial Microcytic Anemia," the "Microcytic Hypochromic Anemia Associated With Splenomegaly and Refractory to Treatment" of Eliel and Bayles,³² "La Syndrome di Cooley nell'Adulto" described by Chini,¹⁷ the "Familial Hemopoietic Disorder in Italian Adolescents and Adults" reported by Wintrobe and associates,⁸⁶ and the observations by Goldhamer³⁷ of characteristic blood changes in several members of 1 family over a span of 3 generations.

ETIOLOGY. The etiology is obscure. Cooley, Witwer and Lee²³ originally believed that the disorder was not primarily hemolytic but rather a disorder of hematopoiesis due to a metabolic fault which also affected bone maturation. Because none of the early reported cases survive puberty, they felt that heredity played no part. Whipple and Bradford^{83b} offered the possibility of an inherited metabolic disorder or a deficiency disease which resulted in disturbed erythropoiesis, faulty bone formation, and bodily development. They compared the lack of a vital factor to that found in pernicious anemia. Lehn-dorff⁴⁷ suggested that the primary disturbance lay in a disturbed bone marrow, which because of some hereditary or "mutational" influence was creating defective red cells.

Impressed with the great similarity of the blood findings in Mediterranean disease and chronic malaria, Nittis and Spiliopulos⁵⁵ suggested that Mediterranean anemia may be a peculiar form of malaria. Eight children ranging in age from 1 to 12 years were treated with quinine. In 2, malarial parasites were found before treatment had been started. Within 3 months after treatment, all had improved clinically and hematologically.⁷

Similar reports from Greece and Italy have stressed the importance of malaria as an etiologic factor, either directly on the child or indirectly through injury of the germ-plasm or fetus in the mother before or during pregnancy.^{3,14,32} Flynn²²

cautions us to remember that malaria is very common in Greece and Italy. The possibility of coincidental infection would be difficult to exclude. Caminopetros^{15a,b} was of the opinion that a coincidental malarial infection in Mediterranean anemia was effective in relieving many of the symptoms.

Smith^{68c} found no history of malaria in the individual members of the 16 families whom he studied. All but 1 of the children and 9 of the affected parents were born in the United States. Fawdry,³⁴ on the basis of his studies on Greeks on Cyprus, concluded that malaria played no part in the causation of the disease, although a few observers in the Mediterranean countries, such as Aravantinos,⁵ prefer to believe that this is an "infectious or parasitic process" with an affinity for the hemopoietic system. No relationship to the Rh factors has been demonstrated.

Damashek^{26b} has suggested, because of the hypochromia, the low mean corpuscular volume, and the failure of response to iron medication, that a disturbance exists in the metabolism of hemoglobin, particularly in development of a normal amount in the cytoplasm of the nucleated red blood cells of the bone marrow. The hyperplasia of the marrow, normoblastic in type, is so extensive in proportion to the evident blood destruction that some cause, other than the constant one arising from the demand to replace the cells lost by fragmentation, is to be sought. Cooley^{21d} postulated either a primary progressive abnormality of the bone marrow or a hyperplasia due to long-continued overstimulation. The presence of large numbers of circulating erythroblasts in some cases has led to the obvious suggestion that these immature cells have escaped into the circulation as the result of chronic demand on a particular type of hyperplastic inadequate bone marrow or because a lowered threshold for the release of erythroblasts occurs as part of the disorder.

The fundamental defect lies obviously in the production of red blood cells. In

the advanced cases the variation in size and shape of the cells is extensive; hypochromia is prominent; the cells contain so little pigment that their destruction yields little jaundice of the serum; and polychromatophilia and reticulocytosis can be demonstrated. The presence of large numbers of stippled cells suggests the possible action of some toxin, but this seems unlikely in view of the hereditary nature of the process.^{26b} More likely the stippling is a manifestation of a defect in structure of the corpuscle.^{68c}

Erythrocytes in every stage of development may be seen. Commonly found are macrocytes with unusual central and peripheral deposits of hemoglobin, the so-called "target cells." (See later discussion of this cell.)

Damashek^{26b} offered as possible cause for the increased hemolysis seen in severe cases: (1) Increased breakdown of hemoglobin precursors which cannot be properly metabolized. (2) A possible increased *in vivo* fragility of the target cells although *in vitro* it has increased resistance to hypotonic salt solution. (3) Some abnormality in the spleen.⁹

Similarly Wintrobe and his associates⁸⁶ suggested that the fundamental defect lay in the production of abnormal red blood cells possessing an adequate or excessive membrane with but little cytoplasmic substance. As a result, the cells withstand the hemolyzing effect of hypotonic solutions more readily than normal cells. Much more fluid needs to be absorbed from a hypotonic solution to produce the spherical shape which is preliminary to bursting. Bradford and Dye¹¹ studied the morphology of the red cells in 8 cases. Wet preparations revealed marked fragmentation. "Knob-like projections were observed to form and to break off from the erythrocytes with regularity." In 2 children, after splenectomy, the cells showed a normal or decreased mean corpuscular volume and an increased mean cell diameter, which suggested cells thinner than normal. Price-Jones distribution curves usually show wide bases and in-

creased coefficients of variations in comparison with normal blood.

Cooley^{21d} believed that the fragmentation and the peculiar, irregular deposits of hemoglobin in the cells are related. The abnormal physico-chemical structure of the cells predisposes them to rapid breakdown in the circulation. This would account for the anisocytosis and poikilocytosis so often observed. The many microcytes seen in the moist film are in part produced by fission of large cells. Valentine and Neel,^{75a} on the basis of their exhaustive original studies and analysis of the recorded cases, have concluded, and apparently rightly, that Mediterranean anemia is due, not to lack of extrinsic iron, but to an inherited inability of the body to utilize or synthesize some substance essential to normal erythropoiesis. "Thus far, efforts to identify the missing factor in thalassemia have been entirely unsuccessful." According to this concept, the difference between the mild and severe forms would be a matter of difference in the capacity to elaborate the unknown deficient substance.

The enlargement of the spleen which appears early is probably due to its overactivity in disposing of the products of fragmentation.^{21d} The spleen shows no evidence of increased erythrophagocytosis. The enlargement of the liver is probably associated with persistent anemia; Whipple and Bradford^{83b} discovered that the liver at postmortem contains about 10 times the amount of iron normally found, whereas the spleen holds less than the normal amount.

After splenectomy, there is a marked rise in the number of nucleated red cells in the peripheral blood. A level of 2200 per 100 white blood cells may be reached.^{21b, 71, 83a, 84} Unlike the transient normoblastic crisis which follows splenectomy in hemolytic icterus and pernicious anemia, the level of nucleated red blood cells continue permanently at an elevated level. It is difficult to explain this, unless the possibility is entertained that the spleen has

previously been actively destroying these cells.

Whipple and Bradford^{83b} pointed out the peculiar pigment distribution in the tissues that could be duplicated in only one other condition, hemochromatosis. The pigments which are chiefly iron-staining are increased throughout the tissues, except in the spleen which contains less than the usual amount of iron. Since the patterns of pigment distribution are almost identical for both hemochromatosis and Mediterranean anemia, these authors concluded that the blood disturbance alone is probably not responsible for the pigment abnormalities in Mediterranean anemia.

Valentine and Neel^{75b} attempt to explain the disturbance in terms of an inherited inability of the body to metabolize completely more than a limited amount of iron into hemoglobin, regardless of the quantity of iron ingested or stored in the body reserves. Thus there would be a deficiency of some factor essential for hemoglobin synthesis, the deficiency being quantitatively much greater in the fatal form of the disease than in the milder genetically related variety. The iron-containing pigments found in the viscera could be explained as being some pigment precursor of hemoglobin which, incapable of being synthesized further, becomes deposited in tissues much as in ochronosis; incompletely metabolized products of tyrosine become deposited in cartilage. Carrying this explanation further, the erythroblastosis becomes the result of the extreme degree of iron deficiency; the bone changes are a response to the markedly hyperplastic bone marrow; and the jaundice, never marked, becomes due to the tendency for all abnormally shaped red cells to be destroyed more rapidly than normal red cells, regardless of the nature of the abnormality.

TARGET CELLS. Haden and Evans^u in 1937 described in fresh preparations of blood of patients with sickle cell anemia red cells which had "a central 'sugar loaf' elevation so that in profile they had the appearance of a Mexican hat instead

of a dumbbell." On stained smear these cells had a peripheral ring and a central dot of hemoglobin with a pale or unstained intermediate zone. The unusual morphology was deemed to be in some way related to the abnormal tendency to hemolysis or the ease of fragmentation. The cells were believed to have an unusual capacity for taking up water so that they almost doubled their volume before hemolysis resulted.

The following year, Barrett⁸ showed that these cells occurred in obstructive jaundice even when the hemoglobin and red counts were normal, after splenectomy, in steatorrhea, and in a group of anemias in which the color index was not more than 0.8. Similar cells were later noted in Laennec's cirrhosis, catarrhal jaundice, metastatic involvement of the liver and cardiac decompensation.⁵⁶

Noting that when viewed from above at different levels these cells presented a "bull's eye" appearance, Barrett⁸ suggested the name "target cell," which was quickly accepted. He showed that the increased resistance to hypotonic solutions associated with the presence of target cells was due not only to the increased resistance of the target cells themselves, but to the tendency of these cells to be associated with other cells of a resistant type. His measurements revealed the target cell to be of slightly smaller diameter ($7.662\ \mu$) than the normal cell ($8.045\ \mu$). He questioned Haden and Evans' description of the cell as "dimpled," and proposed "umbonate" instead, because of the central projections. Studies on wet films revealed that target cells exist in the blood as bowl-shaped precursors. Only on drying does the characteristic "target" appear. In the process of drying bowl-shaped cells form a hump in the center.

Barrett felt, as had Haden and Evans, that the increased resistance of this type of red cell was probably related to the morphology. A decrease in thickness appeared to be the most constant characteristic. He compared the target cell to a bladder which is collapsed and almost

empty. As fluid passes into it, it becomes gradually more distended, but not until the spherical stage is reached will there be any significant increase in the tension of the cell envelope.¹⁶ According to Haden's theory^{40,77} which is essentially a mechanical one, the thicker the cell the greater the fragility and *vice versa*. The precursor of the target cell, the bowl-shaped cell has a surface-volume ratio of relatively high magnitude. In its transformation to the target corpuscle the cell envelope is thrown into folds so that it is larger than that of the disc-shaped corpuscle. This makes for a cell envelope which has a surface relatively large compared to the cell volume.

In 1938 target cells were identified by Bywaters¹³ in a case of Mediterranean anemia. Although not commented on in the text, target cells are evident also in the blood films illustrating Caminopetros' paper (1938)^{15a} on Mediterranean anemia. Since then their presence in this disease has become well recognized. The increased resistance (decreased fragility) was confirmed by Damashek^{26a} and later by Bohrod.^{10a,b} However, in direct contrast to Barrett, Damashek found in his cases that the target cells were larger than the average red cell; he also noted their frequent presence in Mediterranean anemia. Bohrod^{10a,b} believed the target cell to be a young hyperresistant one produced by the bone marrow in response to blood loss, regardless of cause. He found them present in most chronic anemias, and regarded their occurrence as analogous to that of the reticulocyte. Damashek^{26a} regarded the target cell as a congenitally defective cell, probably the fundamental defect in Mediterranean anemia. He pointed out that a cell which is hyperresistant to the *in vitro* hypotonic solution test may nevertheless be hyporesistant to the *in vivo* destructive mechanisms. Cooley^{21c} in his Presidential address before the American Pediatric Society commented likewise that these cells bear no specific relationship to Mediterranean anemia.

Recent evidence indicates that lyso-

lecithin in the relatively stagnant blood of the spleen may increase erythrocyte fragility. Ham and Castle's⁴² studies indicate that the spleen is the chief stasis organ of the body and that as a result of first physical, and later metabolic changes, the red blood cells become spheroidal and more fragile. 'Kniseley^{46a,b} showed in the living animal that blood free of plasma stagnates in the splenic sinusoids for several minutes to several hours. All these studies tend to confirm the opinion that the red blood cells become modified in their passage through the spleen in the direction of increased sphericity and increased hypotonic fragility.

On the basis of the foregoing and Barrett's establishment of a morphologic background (the target cell) for the increased hypotonic resistance of the red blood cells after splenectomy, Singer and his associates⁶⁷ postulate the possibility of a hyposplenic condition in Mediterranean anemia. They noted the appearance of target cells in the blood following splenectomy in dogs and guinea pigs, and ligation of the splenic veins in dogs. They conceive that the erythrocytes when first produced in the bone marrow are relatively thin, but later develop a "normal" degree of thickness on passing through the sinusoids of the spleen. Accordingly, when the spleen is absent, the red cell population tends to be thinner than normal and a definite proportion of red blood cells become sufficiently thin to be recognized as target cells. The possibility is also advanced that the spleen may exert some hormonal influence on the late stages of maturation of the erythrocyte. In the absence of such a hypothetical factor, target cells may develop.

Smith⁶⁸ is similarly convinced that the target cell is non-specific and that decreased fragility does not depend on their presence. Of 53 individuals examined who had a mild form of Mediterranean anemia plus increased resistance to hypotonic solutions, only one-half showed target cells in their blood. Microscopic examination of the sediment in the tubes in the extremely

dilute solutions of sodium chloride often failed to reveal the bowl-shaped precursors of the target cell.

Valentine and Neel^{75a,b} disagree with Vohrod's contention that the target cell is only a young hyperresistant form appearing in the blood as a response to blood loss. They have repeatedly seen evidences of the bone marrow pouring out large numbers of young cells in the absence of target cells. They disagree with Damashek's view that the target cell is the fundamental inherited defect in Mediterranean anemia. They deem the target cell to be a thin, resistant cell, of non-specific nature. They point out that this cell is encountered in many other conditions, that some patients with undoubted Mediterranean anemia fail to display it, and that the number of such cells which appear in a stained blood smear depends to some extent upon the manner of preparation of that smear. They had been able to produce target cells *in vitro* by suspending normal red cells in plasma or serum rendered hypotonic either by the addition of chemicals or by evaporation.

DIAGNOSTIC CRITERIA. The severe form usually has its onset in early infancy, as early as the 3rd or 4th month, though its presence may not be suspected until the 5th or 6th year of age. The first apparent symptom is usually pallor. Anorexia and fatigue are also noted early. A protruding abdomen or palpable spleen may be the first finding to invite further examination.

The anemia is constantly progressive and before many months the disease process becomes well established. Malnutrition and retardation of growth are present in varying degrees. The characteristic facies usually does not become apparent until after the 1st year and sometimes not until several years. The mongoloid appearance results from development of the malar and orbital bones. This causes some prominence and slanting of the eyes. The appearance is heightened by the sallow tinge of the skin. The prominence of the upper teeth are a result of protrusion due to hyperplasia of the maxilla."³³

The abdomen becomes progressively protuberant with increase in size of the liver, and especially the spleen. The spleen may become so weighty that the small child cannot stand up without support lest he be thrown forward. Lymphadenopathy is slight. The scleræ may become mildly icteric. The urine may be dark from urobilin but almost never contains bile. There are no characteristic hemolytic crises but exacerbations may occur during infection.

True cardiac hypertrophy has been demonstrated repeatedly.^{13,57} Hemic murmurs are readily audible and in advanced cases cardiac action may eventually become embarrassed. In the late stages, ecchymosis and free bleeding may be seen. Pathologic fractures are not uncommon. Corcoran²⁴ described an 8 year old boy who first came to medical attention because of a spontaneous fracture of the left femur.

Of more recent interest is the recognition of mild or asymptomatic forms of the disturbance. The mild or "minor" form is beginning to be recognized as widely prevalent, in contrast to the marked or "major" form which is more familiar but comparatively uncommon. (See a later section of this Review for a summary of surveys regarding prevalence.) Careful study of the blood may be the only means by which the mild form may be detected. Subtle roentgenologic bony changes may also be demonstrable.

Well-established cases have anemia as a prominent symptom, but the mild form may have a normal or even increased red cell count.^{26b,86} In both varieties the hemoglobin content is usually lower than the red cell level, giving rise to a low color index. In Smith's^{68c} studies on 54 affected parents and siblings, in 16 families, morphologic changes in the red cells were detected in all individuals regardless of the intensity of the anemia. Smith's description is representative of the current understanding of the red cell abnormalities. "These changes consisted of varying degrees of hypochromic anemia, occa-

sionally polycythemia, an elevated icteric index, the presence of target cells, reticulocytes, macrocytes and stippled red cells, and increased resistance of the red cells to hemolysis. The most common findings, regardless of the severity of the anemia, were anisocytosis and poikilocytosis, basophilic stippling and increased resistance to hypotonic sodium chloride solution. The hypochromic and polychromatophilic macrocytes constituted an important diagnostic feature and were frequently found in predominantly microcytic blood smears." He found a reticulocyte increase ranging from 2 to 8% in all subjects with anemia, the increase being proportional to the severity of the anemia. The number of stippled cells was not always proportional to the number of reticulocytes. Nucleated red cells free in the blood stream were found only with advanced anemia. The vast majority of nucleated red blood cells were normoblasts.

Diversity of red cell size was another prominent feature in Smith's cases. There was a tendency to microcytosis with large cells interspersed. In mild cases the macrocytes were few in number, but in anemic cases they often appeared in great numbers. Smith^{68c} described 3 types of macrocytes. The first is the target cell which is seen in about half of patients with the mild form of the disease. The second is a circular or slightly oval cell having a narrow rim of hemoglobin and a large central achromic area. The third cell, and the one Smith believes to be the most specific, is large and pale and bears its hemoglobin irregularly distributed. This cell looks as if defectively stained. It is extremely thin and leaflike and in wet preparations the edges fold over.

The majority of his mild cases showed a normal or slightly decreased volume of packed red blood cells accompanying the slight to moderate reduction in hemoglobin content. Regardless of severity, anisocytosis and poikilocytosis were invariably present. Smith found, as had Wintrobe⁵⁵ previously, that morphologic changes were far out of proportion to the

mildness of the anemia. Polychromatophilia, Howell-Jolly bodies, Cabot rings, nuclei and reticulated markings appeared frequently.

The fragility test is believed by Smith to be the most important single procedure for recognition of both the mild and the advanced forms. In Mediterranean anemia the span of fragility of the red blood cell is prolonged. In Mild cases unhemolyzed cells may be found in 0.175% solutions of sodium chloride. In severe cases, hemolysis may not be complete until distilled water is used. Angelini⁴ first called attention to the importance of the fragility test in detecting the mild case or carrier. This was confirmed independently by Caminopetros.¹⁵

In the advanced disturbance the leucocytes are usually moderately elevated. One of the patients of Whipple and Bradford^{83a} had a count of 116,000. The leucocytes are usually immature types such as early myelocytes and young lymphocytes.⁵³ Platelets may be normal or reduced. The bleeding and coagulation times are usually within normal limits. The van den Bergh test, if positive, is indirect and the icteric index may be as high as 20. Urine and feces usually show increased amounts of urobilinogen. The serum, calcium, phosphorus and lipids fall within normal limits. In the mild cases no disturbance in the hemopoietic system is usually elicitable apart from morphologic and resistance changes in the red cells.

Resistance to antianemic therapy aids in the diagnosis. When an infant or young child of Mediterranean origin free from infection fails to respond to adequate iron therapy, his future course will probably be that of Mediterranean anemia.^{65c}

Almost always 1 or both parents or grandparents come from one of the Mediterranean countries. The tendency to familial occurrence is of extreme importance in diagnosis. Clinical findings are usually marked only when the anemia is well established and progressive.

Whipple and Bradford⁸³ emphasized the pigment abnormalities in advanced

cases which follow accurately the pattern of pigment distribution in hemochromatosis. Deposition of iron-containing pigments in the skin cause the characteristic subicteric tint.⁵²

The bone marrow shows marked hyperplasia. Parent stem cells of the marrow (hemocytoblasts) are abundant. Myelocytes of all types are numerous as are megakaryocytes. Normoblasts are present in great excess; megaloblasts not so abundantly.^{30,34,78}

Highly characteristic is the increased outpouring of immature red cells which follows and persists after splenectomy. As already mentioned, all observers agree that splenectomy is productive of no clinical improvement apart from the mechanical relief gained by removal of a large and heavy organ from the persistently distended abdomen. The red blood cell count and hemoglobin level appear to remain unchanged after splenectomy.

Little can be added to the observations of Caffey^{14a,b} on the skeletal changes. These findings are essentially non-specific and characteristic of all the chronic hemolytic anemias of infancy and childhood though it is in severe Mediterranean anemia that they are most marked and most frequent. Identical lesions have been seen in chronic malaria, kala-azar and syphilis.⁵⁵ All observers are agreed that the changes in the bones are not primary but secondary to increased blood formation, taking place before the cortex is sufficiently firm to resist the expanding marrow. The extent of skeletal change is usually parallel to the severity of the anemia. Mild anemias have but little bone changes. The later in childhood the disease becomes manifest, the less marked is the osteoporosis.

In the skull, overgrowth of hemopoietic tissue widens the diploic spaces by displacing the outer table externally. The frontal bone becomes the site of the earliest and later of the most marked thickening. The radiating spicules of the frontal and parietal bones may make these bones 5 times their normal thickness.⁴² Skull-

ing of the zygoma cheek bones contributes to the mongoloid appearance. The internal expansion of the swollen upper maxilla sometimes obliterates the maxillary sinuses. Skull thickening may antedate the long bone changes, or *vice versa*.

The long and short tubular bones are uniformly involved, with widened medullary canals, thinned cortices and general osteoporosis. Heavy trabeculations, particularly near the ends of the shaft, may cross irregularly through the medullary spaces. During late childhood and early adult life sclerosis may supervene. Maturation and growth of the skeleton may be retarded. The bone changes come after the blood changes. They have been observed as early as age $4\frac{1}{2}$ months,^{14a} though most of the marked cases have had apparently normal bony contours until after ages 6 to 9 months. Splenectomy appears neither to accelerate nor retard the development of bone lesions.

GENETIC BEHAVIOR. There is little doubt that Mediterranean anemia has a hereditary origin, even though the severe cases usually fail to reach adolescence, and those who have gone on to maturity have failed to procreate.⁵⁰ The question is not whether it is transmitted, but how?

Angelini⁴ supplied early evidence for a hereditary origin. In a study of 26 members of 6 families, he found that nearly all the parents and siblings showed two striking features—a slight bilirubinemia and an increase in the resistance of the red blood cells to hemolysis. The absence of sex linkage is well established although Rundles and Falls⁶⁴ have described a somewhat similar form of anemia thus transmitted.

Caminopetros,^{15a} working at the Pasteur Institute of Athens, found 35 cases of Mediterranean anemia in eastern Greece and made detailed observations on several generations of 4 families, 1 for 4 generations. He demonstrated mild bone changes in apparently healthy members of the family as well as an increased resistance to hypotonic solutions of sodium chloride. He postulated on the basis of

his studies that the disease is transmitted according to the Mendelian law as a recessive character. His study of the family trees brought out the surprising fact that the ancestry of these Greeks included some Chinese who had migrated to Asia Minor. This might account for the "Mongolian eye" occasionally found in some cases.

McIntosh and Wood⁵⁰ concluded that failure to discover a significant excess of marriages of cousins among the cases over that present in the community at large argued against the mechanism of simple recessive inheritance, although the theoretic ratio of 1 affected to 3 unaffected siblings was demonstrated. These authors state that dominant inheritance "may operate to produce Mediterranean anemia in the children of apparently healthy parents in 1 of at least 3 ways: first, by recent mutation of genes as in amaurotic family idiocy; second, by operation of some as yet unrecognized environmental factor which allows the genetic trait to be expressed; third, by association of 2 genes, each of them rare and each capable of producing a recognizable deviation from normal when occurring alone in the parents, but causing an anemic syndrome only when found simultaneously in 1 person." They discount the first 2 hypotheses on the basis that these fail to account for the 1:3 ratio of affected to unaffected siblings, and for the occurrence of the anomaly in twins, and conclude that 2 dominant genes is the most plausible method of transmission. They suggest that the increased resistance of the red cells may be the expression of 1 of these synergistic genes.

Cooley^{22b} believed the inheritance was by a simple dominant. He rejected the idea that the disease was race-limited. "When a disease-producing mutation takes place, it recurs first in the neighborhood of its origin. It is limited to a race in proportion to the clannishness and isolation of the people involved. The appearance in a Negro or Chinese child must not be attributed to an unlikely racial

cross. Such occurrences may be explained on the new appearance of mutations which set up new foci of the disease."

Damashek's⁵⁵ studies on 10 Italian families with target-oval cell syndromes led him to believe that the mild disorder was readily transmitted by a Mendelian dominant, but that the severe disorder required the presence of homozygous genes.

Smith^{65,c} found in every family he studied with a severely affected child that both parents presented abnormalities of the blood, except in 1 family incompletely investigated. Smith suggests that inheritance is by a dominant gene, judging by the family pedigrees of his cases and those of others.^{55,75a,85}

Valentine and Neel^{75a} have made a thoughtful analysis of the manner of inheritance, basing their considerations upon a review of the literature and observations of 34 persons in 4 affected families. In these families 24 persons had hematologic findings qualitatively similar but quantitatively less severe than those of full-blown Mediterranean anemia (which the authors prefer to call thalassemia), including increased resistance of erythrocytes to hypotonic solutions of sodium chloride, target and oval red blood cells, microcytosis, and hypochromia. Valentine and Neel scrutinize the 3 most likely patterns of inheritance. The first is that the severe form results from homozygosity for a factor which when heterozygous produces the mild anemia, *i. e.*, the responsible factor is either an incomplete recessive or a semi-dominant. Otherwise stated, the full-blown disease is inherited as a recessive characteristic, but the heterozygote as well shows significant changes in the blood. This is the theory of Caminopetros¹⁵ and Dameshek.²⁵ The second likely pattern of inheritance is that proposed by Cooley^{21c} and Smith^{65c} that both the severe and the mild conditions have the same genetic basis and are due to a dominant factor variably expressed. Thus 1 person heterozygous for this factor may have a severe disturbance, and another

one only minor changes, the different reactions being determined by environmental and genetic modifiers. The third likely pattern is that suggested by McIntosh and Wood⁵⁰ who proposed that the disease is caused by the simultaneous presence of 2 non-allelomorphic dominant factors, 1 inherited from each parent. After discussing these alternative possibilities Valentine and Neel conclude, from considerations too lengthy to reproduce here, that "the bulk of evidence, with one significant departure, favors the hypothesis that the mild state is due to heterozygosity for a factor which when homozygous results in full-blown thalassemia." The authors present this view as the working approach which fits the known facts best, even though certain trends remain puzzling, such as the tendency for a significant excess of mildly affected individuals among the offspring of mildly anemic and normal parents. Technically they favor the theory of an incomplete recessive gene, although Gates^{56a} from a review of the same series of cases inclines toward the view that the gene is a partial dominant, sublethal when homozygous.

PREVALENCE AND DISTRIBUTION. The disease is largely confined to the population living along the Mediterranean littoral and their descendants. Many writers confine the disease to the northern shore, but it has been reported from both shores.³⁰ Most of the patients come from Greece, Sicily, and Calabria. Strangely enough, the first case from Italy was not reported until 1934,⁶² almost 10 years after Cooley's original description. Many believe that the southern Italians with the disease are partly of Greek origin.²⁷ During the height of the Greek era of civilization and particularly during the reign of Alexander the Great, there was extensive colonization of all the Mediterranean countries by Greece, with subsequent intermarriage. Fawdry³⁴ found many advanced cases among the Greek inhabitants of Cyprus; in 2½ years as medical officer in 1 district he encountered 20 such patients, 19 children and 1 adult.

Investigation of the fossilized bones of the mound builders of eastern Arkansas,⁸¹ and of the pre-Columbian Peruvians⁶ indicate that in those past civilizations a disease existed whose roentgenologic findings are identical with Mediterranean anemia.⁸⁷

A number of instances of an identical or closely similar anemia have been reported in non-Mediterranean races. Saracoglu⁶⁵ described Mediterranean anemia in a 9 year old Turkish boy whose brother had died from a severe anemia. This is the fourth reported case among individuals of Turkish descent. Sendrail *et al.*⁶⁶ observed a Spanish case. Bywaters¹³ described a 10 year old ill child with a blue-eyed English policeman as father, an English mother, and 4 healthy siblings.

Diwani³⁰ found the disease in 2 Egyptian children. Mukherji⁵⁴ has recorded a 30 month old Hindu male with this anemia. Patel and Bhende⁶⁰ noted a Brahmin boy who had the disease complicated by cirrhosis of the liver and kyphosis. Coelho¹⁹ has observed 2 Indian girls aged 6 and 9 years, and Napier⁵⁵ and his associates a Hindu boy aged 10 years. Dhayagude²⁹ has described a 7 year old Brahmin boy who had the disease in a moderately severe form, and from New Delhi, Malhotra and Chhuttani⁴⁹ have reported a 4 year old affected child.

The first case in a Chinese child was reported in 1940 by Foster.³⁶ No Roentgen rays were taken of the skull or long bones. The mother had hematologic findings suggestive of a mild form of the disease. Wang and Khoo⁸² in the same year report 3 cases of hemolytic anemia with erythroblastosis in Chinese children. One of the 3 appeared to have Mediterranean anemia.

Damashek^{26b} in his article on the "Target Cell Syndrome" (1943) mentions a typical case of Mediterranean anemia in a Negro child observed at the Mt. Sinai Hospital of New York. Stiles, Marlowe and Dangerfield⁶⁹ describe a 33 year old Negro with the hematologic findings of the disease; this individual's mother and

sister also had histories of anemia and a maternal great-grandparent was of Italian origin. Faber and Roth³³ report an instance in a Negro girl. Damashek,^{26c} who has often seen target and oval cells in Negroes with no evidence of sickling, states the entire subject needs amplification. Downey³¹ feels that many cases in other races may have been overlooked because the symptoms are sought only among Mediterranean descendants.

Neel and Valentine⁵⁶ made a city-wide survey of the number of individuals of Italian descent living in Rochester, N. Y. and the prevalence of Mediterranean anemia among them. These individuals for the most part were immigrants or descendants of immigrants from Sicily or southern Italy. Survey of hospital records revealed the occurrence of the severe form of the anemia was 1 per 2368. From this datum they estimated the number of mild cases which might be present among this same population, basing their calculation upon the hypothesis that severe Mediterranean anemia is an inherited disorder due to homozygosity for an inherited factor, whereas the milder disorder is due to heterozygosity for the same factor. Their calculation led to the ratio 1 per 25 adult Italians for the frequency of the minor disorder in Rochester's population. This estimate has thus far not been validated by direct study of representative samplings.

PROGNOSIS. The prognosis is contingent on the fact that the disease shows a wide range of variation, from the "trait" or carrier state to the advanced form marked by severe anemia. Individuals having the mild form may and usually do have a normal span of life, whereas individuals having the severe form, with onset early in life, marked anemia, splenomegaly, and bony changes, rarely survive adolescence. They succumb from profound anemia or intercurrent infection. Very occasionally, a temporary remission may be induced by transfusion or splenectomy. Less often a more or less permanent

remission takes place at blood levels compatible with moderate normal activity.

The number of reports keeps growing of patients with moderately severe anemia and marked hematologic abnormalities with or without clinical and roentgenologic findings who have survived adolescence. Mandeville⁴⁸ described a 15 year old with an advanced roentgenologic picture. No mention is made of any associated findings. In 1936, a 24 year old Italian pharmacist with pallor, jaundice and splenomegaly who had a normoblastic crisis after splenectomy was reported by Allen and Childs.² Dalla Volta's²⁵ patient, aged 30, showed considerable excess pigment in his organs and large cells suggesting the foam cells described by Whipple and Bradford.^{83a} Caminopetros^{15a} listed 2 adults with Mediterranean anemia and Aravaninos⁵ 3 aged 16, 21 and 22 years. Thalheimer⁷⁴ reported a 14 year old male whose illness dated from 1 year of age, and added a note regarding a 16 year old patient mentioned to him by Dr. Benjamin Kramer of the Jewish Hospital of Brooklyn. Atkinson⁶ followed 2 adult siblings of Greek parentage, a brother and sister, 17 and 20 years old, with characteristic findings, for a period of 12 years. A Sicilian youth of 20 with splenomegaly and a hypochromic microcytic anemia resistant to therapy was seen by Win-trobe;⁸⁶ since the age of 10 this patient had presented a degree of hypochromia and poikilocytosis out of all proportion to the anemia. Eliel and Bayles³² described a 39 year old American born Italian woman who had a hypochromic, microcytic anemia with splenomegaly, subclinical jaundice, and blood change characteristic of the Mediterranean anemia of childhood. Fawdry³⁴ reported an adult Greek male on Cyprus, 20 years old, who had stunted growth, cardiac enlargement and a mongoloid facies. Smith's^{68b} 19 year old boy was first described in 1942. He had been followed since the age of 4 and was last transfused in 1929. A younger brother, aged 8; also with Mediterranean anemia, is being treated symptomatically

without transfusion. Van Ravenswaay's⁷⁶ patient who practised forms of treatment quite primitive was 35 years old. The 20 year old male described by Voorhies and Sloan⁸⁰ demonstrated a characteristic hematologic picture, mongoloid facies and extensive bony changes. Read's 36 year old male apparently had a mild form of Mediterranean anemia.

Of course, now that the great reservoir of mild cases has been discovered, distinction must be made between the mild and severe, or "minor" and "major" forms of the disorder when adult patients are being discussed.

TREATMENT. There is very little that has been left untried in the treatment of Mediterranean anemia. Feeding of various liver and splenic fractions have given negative results. Adrenal cortical extract has been tried without success. Anterior lobe of the pituitary, thymus and bone marrow extracts failed to modify the clinical picture. Vitamin concentrates, raw pancreas, predigested beef, and pentanucleotide have proven of little avail.^{83b} No specific corrective or prophylactic treatment is known. All therapy must be directed toward preventing complications and offering symptomatic relief. This anemia is almost the only exception to the dictum that a hypochromic anemia always responds to iron therapy.^{26b}

Hunter,⁴⁴ in an attempt to depress the hemopoietic organs so that escape of immature nucleated erythrocytes into the blood stream might be prevented, gave Roentgen ray in dosages in excess of the optimal. Acute bone marrow depression was produced. Aided by transfusions, the platelets and white blood cells returned to normal levels. After several weeks, the red cell count rose to a higher level than had been observed at any time prior to the irradiation. The clinical condition also showed improvement. No reports of further follow-up studies were published. Wang and Khoo⁸² tried Roentgen ray therapy with 1 of their patients. There was a marked depression of all blood elements including the platelets. Daily irra-

diation of the spleen of a 9 month old patient by Amesse and Barber³ offered no relief and was discontinued when the patient became worse.

Nittis and Spiliopulos,⁵⁸ noting a similarity in the blood findings in Mediterranean anemia and chronic malaria, attempted intensive antimalarial therapy in 8 patients having the advanced picture of Mediterranean anemia. Seven of the 8 showed clinical and hematologic improvement 3 months after therapy. In direct contrast, Caminopetros,^{15a} observing the benefit which occurred in a thalassemic patient who developed a coincidental malarial infection, inoculated 7 other patients with the disease. His results led him to state that this therapy was as effective as is Roentgen ray therapy in leukemia.

Goldman and Malavazos,³⁸ taking into account Whipple and Bradford's opinion that the basis of the pathologic process lay in a vitamin or endocrine disturbance, tried pregnancy urine and vitamin B₆ with 3 patients. All responded with bone marrow hyperplasia of the erythrogenic series, and moderate increase in the red cell count in the peripheral blood; but as treatment was continued beyond 4 weeks a fall in the count took place.

Van Ravenswaay *et al.*⁷⁶ described a primitive treatment practised by a 35 year old patient in his youth in a small town in Greece. (This patient had 3 children who died of Mediterranean anemia.) Four to 6 folds of skin would be lifted over the liver or spleen and each fold would be nicked with a knife. Then a tumbler was held over this area with a lighted piece of cotton placed inside. This consumed the oxygen and created a suction. The tumblers were left in place until they loosened themselves which was usually when one-third full of blood. As many as 4 to 5 tumblers would be used at one time. Native children have also been taught to lean over a sharp piece of furniture such as a table and compress the spleen for as long as they could stand the pain. Improvements in breathing would follow,

and the patients would repeat this procedure 6 to 8 times per day.

Splenectomy is of no great value.^{1,2,4,73,84} It is practised only in patients with marked weightiness of the organ or mechanical obstruction. Very occasionally a temporary remission will follow splenectomy.

Blood transfusions are without permanent benefit.⁶¹ Nevertheless, periodic transfusions should be given to maintain blood levels compatible with normal activity. Koteen and Brooks⁴⁵ have set up an out-patient transfusion clinic for patients with this disorder. Adequate levels of hemoglobin and red cells are maintained by whole blood transfusions every 2 weeks. Transfusion maintains the hemoglobin and erythrocytes on low but constant levels. When the levels begin to drop more transfusions are required. Transfusion reactions though frequent are usually not serious. Only with the aid of repeated transfusions have children with severe Mediterranean anemia lived to and beyond adolescence. The 19 year old thalassemic described by Smith^{68b} is an exception. Valentine and Neel^{75a} caution against using blood for these patients drawn from the parents or relatives because: "1. Frequently the donor's blood is significantly hypochromic, so that the hemoglobin content per 100 cc. of blood is below the desired optimum. 2. More important, erythrocytes which have the same type of defect inherent in the erythrocytes of the recipient, albeit quantitatively less severe, are being transfused. There is presumptive evidence that such cells are undergoing more rapid destruction than normal erythrocytes, in the body of both the carrier, who is the donor, and the thalassemic recipient. 3. It is undesirable to bleed donors whose hemopoietic apparatus is already overstrained and defective. It seems likely that the supportive value of such transfusions may be significantly reduced. Transfusion from an extraneous normal donor or from a proved normal relative is a more rational procedure."

Conclusions. The observations summarized in the preceding pages represent

a body of information impressively elaborate when one considers that the disease Mediterranean anemia was not recognized as an entity until 2 decades ago. Numerous problems related to it are as poorly understood now as when Cooley and Lee made their first report. The basic defect has an inherited origin, and probably is more closely related to red cell than to hemoglobin formation (though both seem defective in advanced cases), but what is its precise nature? The recognition of the carrier state represents a major step forward, of as great importance to adult as to pediatric medicine, yet the mechanism of genetic transmission has become more rather than less obscure as a consequence of this discovery. Valentine and

Neel's estimate of 1 affected carrier per 25 individuals of Italian origin clamors for corroboration. We must have adequate knowledge regarding heredity in order to be able to give eugenic advice to young couples when 1 or both are found to carry the trait. Treatment, of course, has always been highly unsatisfactory, palliative transfusion being the only helpful procedure known. Widespread attention should be given to Koteen and Brooks' experience in an out-patient transfusion clinic. If transfusions can be given to active cases in out-patient departments at frequent regular intervals, the rate of infections will decrease, the costs to hospitals will diminish and the children themselves will fare better.

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GYNECOLOGY AND OBSTETRICS

UNDER THE CHARGE OF

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OVARIAN TUMORS

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TUMORS originating in the ovary continue to be of great interest to the clinician as well as the gynecologic pathologist, judging by the great number of papers which have been published in recent years. To the clinician, once the diagnosis of an ovarian tumor has been made, the chief interest lies in knowing whether or not it is malignant. To the pathologist, however, the details of structure, origin and classification carry the study into much finer ramifications. For those who are interested in this highly specialized field of gynecologic pathology, the recent collective review of Dockerty⁶ will be of great value. While the review which is here presented will overlap Dockerty's at some points, it will stress the clinical rather than the pathologic aspect.

GRANULOSA CELL TUMOR. This tumor, which produces estrogenic hormone, is being more frequently recognized each year. In a study of 21 cases Henderson¹¹ found that they presented considerable variation in gross and microscopic appearance. The smallest tumor was microscopic in size while the largest weighed 24 pounds. The moderate sized tumors, as a rule, revealed areas of interstitial hemorrhage, cystic degeneration and necrosis. The solid portions were whitish in color and of brainlike consistency. Marked friability was a constant feature of those tumors which were predominantly of the follicular type. The common clinical evidence of hormone activity was uterine bleeding

alternating with irregular periods of amenorrhea. Amenorrhea of 3 to 4 months duration was common while periods as long as 2 years were occasionally observed. When such long periods of amenorrhea occurred during the age period of the menopause it was often difficult to determine whether the amenorrhea was due to a physiologic menopause or to the tumor. Endometrial hyperplasia of the "Swiss-cheese" type was the common pathologic evidence of excessive estrogen secretion. During the postmenopausal years, myometrial hypertrophy frequently accompanied the endometrial hyperplasia while, in the 1 case occurring before puberty, secondary sex characteristics were precociously developed. Evidence of biologic activity of the tumor was present in 15 of the 21 cases. In 10 cases the evidence was endometrial hyperplasia and in 5 it was a clinical history of irregular uterine bleeding. There were 5 cases of uterine fibroids as well as 2 cases of carcinoma of the endometrium. Until more is known about the malignant potentialities of the granulosa cell tumor and the effect of irradiation on a large number of cases has been observed it would seem wise to advise postoperative irradiation in all cases in which the tumor occurs at the time of, or after, the menopause. Considerable variation in the rate of growth of these neoplasms was evident. One patient was observed for 3½ years without any increase in size of the neoplasm, while the largest

tumor in the series occurred in a patient whose only complaint was a rapidly enlarging abdomen for 1 year. Another case demonstrated marked rapidity of growth following the intrauterine application of radium for metrorrhagia.

At the Mayo Clinic, according to Hodgson, Dockerty and Mussey,¹² between 1910 and 1944 there were 62 granulosa cell tumors removed. This constitutes 1.63 % of 3800 ovarian tumors encountered during this period. About 60 % of these tumors were found in women who had passed the menopause. The most common clinical symptoms were uterine bleeding (74 % of 62 cases), amenorrhea (22 %) and abdominal enlargement (29 %). From a study of the records it was apparent that the tumors that produced these symptoms grew slowly and might have been present for as long as 35 years. Evidence of hyperestrinism is afforded in this series by symptoms of precocious puberty, amenorrhea and postmenopausal bleeding, and is supported by the incidence of adenomyosis and endometriosis (9.6 %), which often occurred in postmenopausal patients; uterine fibromyomas 51.6 %, uterine hypertrophy 59.6 % and proliferative endometrium 67 %. Differentiative (secretory) changes in the endometrium were correlated in 3 cases with luteinization in the granulosa cell tumor. Luteinization without the expected endometrial change was observed in 12 additional tumors. Thus the deduction that granulosa cell tumors may produce the differentiative hormone, progesterone, is suggested. The high incidence (21 %) of endometrial carcinoma observed in 38 postmenopausal patients who had granulosa cell tumor was remarkable. In 3 of the 8 cases, carcinoma of the breast with axillary metastasis also developed. This phenomenon of coëxistent ovarian, endometrial and mammary carcinoma in the human being bears a marked similarity to the results of experiments on laboratory animals in which estrogen stimulation appears to be a factor in carcinogenesis. In this group of 62 cases of granulosa cell

tumor there were 4 definite recurrences and 1 possible recurrence. Conservative surgical procedures among the postmenopausal patients accounted for 4 and possibly 5 failures to obtain good results. Thus 4 recurrences and 3 deaths occurred after conservative operations in the postmenopausal group. This appreciable incidence of recurrences among older patients indicated that bilateral oöphorectomy with hysterectomy is advisable for granulosa cell tumors affecting women past middle life. Less radical procedures, on the other hand, seem to be indicated for granulosa cell tumor affecting younger women, inasmuch as no recurrences were noted and pregnancy subsequently occurred in 3 cases.

In regard to the curability of granulosa cell tumors, Jones and Te Linde¹⁴ of the Department of Gynecology at Johns Hopkins Hospital have found that as these tumors become more commonly recognized, it is apparent that the prognosis is more difficult to determine than formerly believed. It was at first thought that these tumors were benign. As the number of case reports multiplied and the follow-up studies were available, it became evident that the granulosa cell tumor, although more benign than most other ovarian carcinomas, nevertheless carried with it a definite malignancy. The difficulty in the prognosis of the granulosa cell tumor is further reflected by the wide variation of statements in the literature concerning its malignancy. The occurrence of malignancy among the granulosa cell tumors is quoted by various authors as being as low as 10 % and as high as 55 %, with all intermediate gradations. The collection of adequate statistics on the malignancy of the granulosa cell tumor is difficult as the incidence of late recurrence in this tumor group makes 5 or even 10 year follow-up studies inadequate. This fact has been strikingly brought to their attention by the recent return of 3 patients to the gynecologic clinic, all having recurrent granulosa cell tumors 15, 16 and 19 years, respectively, after their original operation. All 3 pa-

tients have died, 18, 20 and 21 years, respectively, following the removal of the primary tumor, in spite of the fact that the tumors were well encapsulated and showed no evidence of metastasis or implantation at the original operation. One case recurred in spite of a bilateral salpingo-oophorectomy and a hysterectomy. One case with widespread inoperable abdominal metastases, responded well to deep Roentgen ray therapy over a period of 3 years but a subsequent recurrence of the growth proved refractory to treatment. Total urinary estrogen values were not extremely high in 2 cases but within the range of values for normal cyclic women. However, the values were well above those usually found for postmenopausal women, into which age group all 3 patients fell at the time of recurrence. All 3 cases exhibited clinical signs of estrogenic activity on recurrence of the tumor.

THECA CELL TUMORS. Although it is less than 15 years since theca cell tumors of the ovary have been accurately described, they have attracted considerable attention. In analyzing 23 of these tumors Banner and Dockerty¹ state that the tumor probably arises from certain pluripotential mesenchymal cells of the ovarian cortex and is related on the one hand to the histologically mature fibroma and on the other to the comparatively dedifferentiated granulosa cell neoplasm. Controversy still exists, however, in regard to certain clinical, pathologic and physiologic aspects of the tumor and further statistical data are necessary to elucidate some of these issues. In their series theca cell tumor appeared to be relatively rare. It comprised about 3% of the group of solid ovarian tumors, was about a third as common as granulosa cell tumor, and was less than a tenth as common as ovarian fibroma. The average age of patients who had theca cell tumors was about 54 years; 65% of the patients were 50 years of age or older. Extremes of 80 and 26 years were noted. Theca cell tumors have not been reported as occurring prior to the age of puberty. Pregnancy had oc-

curred at least once in 80% of the patients. Irregular vaginal bleeding of the postmenopausal type or gross menstrual irregularities of younger patients existed in more than 60% of the reviewed cases. The largest tumor measured 12 cm. in its greatest diameter and the smallest 3 mm. The average diameter was about 6 cm., the actual size of the tumor bearing no apparent direct relation to the degree of clinical "function" as measured by the duration or the severity of associated menstrual disturbances. The color of the tumors varied considerably. The outer aspect, usually smooth and often bosselated, presented tints ranging from brownish gray or even pearly to a dull orange yellow. This latter color was nearly always apparent somewhere on cut section of the tumor. The consistency was in general firm but rubbery rather than densely hard and inelastic. Degenerative central cysts were present in several of the larger tumors and these cysts appeared to arise through chronic infarction and edema as a result of twisting of the pedicle of the tumor. Residual ovarian tissue could be found in the capsules of all but the larger neoplasms. A smooth glistening capsule invested all but 2 of the tumors, and these 2 appeared to be fixed by adhesions to neighboring structures. These tumors appear to be derived from the ovarian mesenchyma and they are closely related to the granulosa cell group of tumors. Occasionally the relation may be a histologic one with elements of both types discernible within the same neoplasm. Usually, however, both tumors present "purity" of type. The relatively high incidence, especially in postmenopausal patients, of associated myometrial hypertrophy and uterine fibromyomas, combined frequently with endometrial hyperplasia and cervical and fundal carcinoma, suggests the production by the ovarian tumors of estrogenic hormone. Inability to demonstrate measurable quantities of this hormone through assays of tumor tissue is perhaps not to be regarded as conclusive evidence of absence of the

hormone. Although the tumors did not invariably produce clear-cut clinical symptoms, their presence could often be suspected by an alteration in the menstrual habits of the patient. Postmenopausal bleeding from a hypertrophic endometrium was commonly encountered in these patients.

In reporting 4 cases of theca cell tumors McGoldrick and Lapp²⁰ have reviewed the subject and state that not infrequently there are no menstrual abnormalities noted. Varying degrees of virilism have been reported in 3 instances. Atypical bleeding may be cyclic or irregular, scanty or profuse. Other changes noted are a rejuvenation of atrophic breast tissue, recrudescence of libido, and a revitalization of the vaginal mucosa. Physiologically, because of the hyperestrinism produced by the tumor, the uterus becomes enlarged and softened with glandular cystic or adenomatoid hyperplasia of the endometrium. All symptoms abate upon extirpation of the tumor. In the premenopausal group, the periods again recur regularly; in the postmenopausal group, the bleeding ceases. They state that the tumor is always unilateral, no bilateral theca cell tumors ever having been reported. Thecoma tends to simulate the fibroma of the ovary in size, shape and consistency. A capsule is commonly present. On cut section, the surface is noted to be composed of varying sized islands having a diagnostic yellow hue and separated by grayish white fibrous bands. Small cystic areas are an inconstant feature and result from liquefaction necrosis. At the most, theca cell tumors are a form of low grade ovarian malignancy. Most surgeons feel that treatment need not be radical, a simple excision of the ovary being sufficient in the vast majority of cases. Theca cells are considered to be radioresistant, so that radiation therapy is generally considered to be of little or no value except for the diminution of the size of the tumor by the effect of Roentgen ray on the fibrous tissue stromal elements.

ARRHENOBLASTOMA. While the granulosa cell and theca cell tumors may be called feminizing tumors because they secrete female sex hormones, the arrhenoblastoma is a masculinizing tumor and is a much rarer tumor than the others. In presenting a case of this type Kanter and Klawans¹⁶ state that when virilism exists in the female, several conditions must be considered in differential diagnosis since adrenal and pituitary disease can both cause the condition. Cushing's syndrome, basophilic adenoma of the pituitary, is characterized by hypertrichosis and amenorrhea but no hypertrophy of the clitoris or larynx and no ovarian tumor. On the other hand, there are hypertension, glycosuria, obesity of face, neck and trunk, acrocyanosis and interference with visual fields. The adrenogenital syndrome may be quite difficult to differentiate from arrhenoblastoma since most of the symptoms of one are present in the other. The presence of an ovarian tumor would point to arrhenoblastoma but the tumor may be so small that it cannot be palpated. The adrenal may be visualized by Roentgen ray after perirenal air insufflation, but here also the tumor may be too small to be visualized. Careful blood and urine studies may give a clue to the diagnosis. Adrenal tumors are associated with hypertension, a decrease in serum sodium and an increase in serum potassium. The urinary sodium is increased to 3 times normal and the potassium in urine is decreased.

In a study of 70 cases collected from the literature, which were all the cases available up to the time of their report, Krock and Wolferman¹⁷ have gleaned several interesting observations. The conclusions of investigators on sex reversal, that the right ovary exhibits testicular transformation more often than the left in animals, is not borne out in the human, since the tumor was found on the left side in 31 cases, and in 2 it was stated as occurring on both sides. It is also to be noted that the histologic pictures, as reported, lack the necessary degree of uniformity to be convincing that this unusual

tumor is a distinct pathologic entity. Alteration of secondary characteristics in the female is not limited to one particular group of ovarian tumors. There are many reports of masculinizing ovarian tumors in which histologic examination has failed to disclose the presence of testicular elements. In other words, we have, on one hand, the so-called "arrhenoblastoma" with a variable picture of masculinization clinically but with what apparently are testicular elements histologically, and, on the other hand, a number of different types of ovarian neoplasms in which the predominant tissue is other than testicular, but with clinical virilism. The one thing common to 2 such widely divergent pictures is the presence of tridermal tissue elements, which can be demonstrated by routine studies in approximately one-third of the cases. To create a new pathologic entity, based upon the predominant tissue present, as has been done with arrhenoblastomata, would lead to hopeless confusion. In the final analysis, our present knowledge is still deficient concerning the exact origin of all the factors controlling the development of secondary sexual characteristics. Upon this basis, it is their plea that all masculinizing ovarian tumors be classified as teratomata with a one-sided development, be such development testicular elements (either in the form of interstitial cells or seminiferous tubules), adrenal cortical remnants, or even other tissues or their anlagen whose rôles at present under normal conditions, in the development of the secondary sexual characteristics of the individual, are not fully understood. Or we may retain the term "arrhenoblastoma" to indicate such a "clinicopathologic" condition, rather than regard it as representing one constant pathologic entity to be pigeon-holed thus by any pathologist inspecting the tissue microscopically.

A most interesting and unusual case of arrhenoblastoma, complicating pregnancy, probably the first instance of its kind has been reported by Brentnall.² The reason for the extreme rarity of the 2 conditions

in the same patient is that once a tumor has produced its first symptoms, amenorrhea, the woman is sterile but usually several months must pass before the symptoms of virilism appear. There is, therefore, only a very short period during which pregnancy and virilism can synchronize. This patient was delivered by Cesarean operation and it is of interest to note that the child was normal except for the external genital organs which were those of a pseudohermaphrodite.

The term *gynandroblastoma* was first used by Robert Meyer in 1930 in a case of arrhenoblastoma which had in part a histologic similarity to granulosa cell tumor and was accompanied by uterine hypertrophy. He suggested that ovarian tumors may arise from indifferent elements which become morphologically and functionally hermaphroditic so that they may cause both hypertrophy of the uterus and masculinization. In reporting a case of their own and after reviewing the literature, Mechler and Black²¹ state that ovarian tumors exist in which there is alteration of the secondary sexual characters of the bearer in the male direction, presumably as a result of the androgenic hormone liberated by the tumor; but at the same time there is a continuation of cyclic menstrual bleeding, with indications of hyperestrinism. Such cases differ from granulosa cell tumors both biologically and histologically. There is no constant cell pattern in these cases, and they appear to represent combinations of granulosa cell tumors and arrhenoblastomas. The presence of tubular structures lined by cylindrical, mucus-secreting epithelium has been noted. Interstitial cells carrying lipoids, resembling the testicular Leydig cells, are common to both gynandroblastomas and arrhenoblastomas. There is presumptive evidence that these cells are the source of the androgenic hormone. The question of antagonism between the androgenic and estrogenic hormones in gynandroblastomas is complex. Androgenic hormones are capable of inhibiting menstruation in the normal woman, theo-

retically by inhibiting the gonadotrophic factor of the hypophysis, thereby preventing ovarian formation of estrin. This does not apply in case of the tumor, necessarily, as the tumor cells may be able to produce estrin independently of hypophyseal factors. Therefore, hyperestrinism and virilism may coexist in the same person. The term gynandroblastoma may be usefully applied to describe a clinical-pathologic syndrome, but there is no constant accompanying histologic pattern. The ovarian tumor concerned has epithelium-lined tubules and interstitial cell groups in common with arrhenoblastoma. The impression is gained that combinations of the granulosa cell tumor with arrhenoblastoma do occur but that one element or the other usually predominates to the extent that the double biologic effect is lacking. It is suggested also that the gynandroblastomas are teratomatous.

DYSGERMINOMA. In contrast to the previous tumors which we have considered, the dysgerminoma elaborates no sex hormones and is the tumor most frequently associated with pseudohermaphrodites and women with poorly developed genitalia. In presenting a case of this type, Long, Ziskind and Storck¹⁹ comment that while a large pelvic tumor in a pseudohermaphrodite or in a female with infantile genitalia suggests dysgerminoma, the diagnosis is not so easily made in those women who have normal secondary sex characteristics. Aside from symptoms referable to a disturbance of hormonal balance, the complaints and findings are usually those due to a large and rapidly growing pelvic tumor. Changes in the menstrual period, abdominal pain and enlargement are the common complaints. The tumor, as a rule, enlarges rapidly, attaining a size sufficient to fill the lower abdomen in 3 to 6 months. Degeneration of the fast growing mass frequently occurs and is sometimes responsible for fever, night-sweats and leukocytosis up to 20,000 cells per c.mm. Other more infrequent symptoms, some of which are due to pressure, others to malignant extension, are renal

colic, urinary frequency, respiratory embarrassment, diarrhea, vomiting, ascites, anemia and loss of weight. The tumor is one of childhood and young adulthood, frequently having its onset early in puberty. In this characteristic it varies widely from its homologue, the testicular seminoma, which rarely is found before the 30th year. The diagnosis rests largely upon a consideration of the duration of symptoms, the age of the patient, state of sexual development, and the presence of a rapidly growing pelvic neoplasm. Palpation of the abdomen and pelvic examination reveal a hard, elastic mass with an irregular, nodular surface. Occasionally the Roentgen ray may be able to differentiate between a teratoma or dermoid cyst from a dysgerminoma by demonstrating the presence of calcium in the tumor. Opinions as to the incidence of malignancy in dysgerminomas vary. As yet there are such few cases on record and each writer's experience is so small that no clear conception of the degree of malignancy has been gained. The pathologist is able to add little to the problem's solution, for histologically all dysgerminomas display malignant features, and there appears to be no constant relation between these and the degree of malignancy. At present the most valuable criteria of the malignancy of individual tumors are clinical. If at operation a unilateral tumor is found which has no obvious extension, as indicated by the absence of infiltration of adjacent structures and regional lymph node involvement, it may be regarded as benign until proved otherwise by the clinical course. Infiltration of the broad ligament, uterus, opposite ovary, rectum and bladder, and lymphatic spread to the retroperitoneal lymph nodes occur in a definite proportion of cases, but metastases to distant organs are rare. The importance of accuracy in estimating the degree of clinical malignancy at the time of the operation is self-evident when the occurrence of dysgerminomas in young individuals is placed in relation to the effective methods of treatment of the malignant phase, viz.,

radical surgery and deep Roentgen ray therapy. The price paid by the patient for this form of treatment is removal or destruction of both ovaries. While this fate is of little concern to the pseudo-hermaphrodite or to the patient with marked hypogonadism, it is undesired by the young girl who has not yet developed secondary sex characteristics and by the young woman who desires to bear children. Simple extirpation has come to be considered good judgment on the part of the surgeon who finds at operation a solid, non-adherent, ovarian tumor. Should gross and histologic study prove the mass to be a dysgerminoma, frequent follow-up examinations should be made in an effort to discover early any possible recurrence of the neoplasm, which when found should then be treated by irradiation. If, on the other hand, there is present at operation evidence of local extension, a more radical surgical procedure should be adopted and followed after operation by deep Roentgen ray therapy. In inoperable cases, thorough irradiation alone remains the treatment of choice. Those patients whose advanced dysgerminomas are attacked early are offered an excellent prognosis. The cut section presents a homogeneous, friable, glistening, brainlike surface through which run scanty fibrous septa. Areas of yellowish discoloration indicate lipoid degeneration, and frequently this has progressed to the stage of frank necrosis with the formation of broad zones of necrotic tissue and hemorrhagic cysts of varying size. The microscopic appearance of sections of dysgerminoma is one of the most characteristic found among solid ovarian tumors. The tumor cells are large and round with hyperchromatic centrally placed nuclei containing large nucleoli, and a narrow lightly granular cytoplasm. Frequent mitotic figures appear. The cells are loosely arranged in column or islands, frequently described as alveolar, separated by varying amounts of loose, edematous, poorly vascularized connective tissue. Degenerative changes and hemorrhages are com-

mon. The connective tissue nearly always is diffusely infiltrated with lymphocytes, and these with the presence of small epithelioid cells and large giant cells create a picture simulating tuberculosis.

BRENNER TUMOR. This is another of the rare ovarian tumors which is usually unilateral. In reporting a case of bilateral Brenner tumors Johnson and Dockerty¹³ remind us that the Brenner tumor comprises 2% of solid ovarian tumors, which in turn comprise only 20% of all ovarian tumors. Brenner tumors may be divided into 2 groups, namely, (1) a completely solid tumor (70%) and (2) a solid tumor in the wall of a cyst, usually a pseudomucinous cystadenoma (30%). Brenner tumors vary in size from a microscopic nodule to a huge growth almost filling the abdominal cavity. Small tumors cause no symptoms, being discovered at necropsy. The majority of Brenner tumors are found after the menopause. They never occur before the onset of puberty. In their series of 20 cases, the ages of the patient varied from 34 to 75 years. Although uterine bleeding has been present in a number of reported cases, there is no proof that this bleeding is on a hormonal basis. The most common complaint is of an abdominal tumor. In all cases reported in the literature the tumor was benign, but these growths frequently are associated with other neoplasms of the genital tract. Macroscopically, Brenner tumors resemble ovarian fibromas, being whitish in color, dense and hard. Microscopically, the picture is uniform. There are islands of squamous-like epithelial cells in a dense stroma of fibrous tissue. The peripheral rows of these cells frequently are arranged in palisades. Some of the islands are cystic with an inner lining of columnar, mucus-producing cells. Mitotic figures are not observed. These tumors are to be distinguished from granulosa cell tumor and from metastatic squamous cell epithelioma. In any solid ovarian tumor, especially of the bilateral variety, the possibility of Krukenberg's tumor should be

kept in mind and exploration of the upper part of the abdomen should be undertaken. Johnson and Dockerty state that there are 3 fairly plausible hypotheses of origin. The first is that of Robert Meyer who reported that Brenner tumors had been derived from Walthard's cell rests which frequently are found in the cortical and medullary zones of normal ovaries. These rests undergo metaplasia to form Brenner tumors. However, these rests often are found in the mesosalpinx and underneath the serosa of the fallopian tubes, locations in which Brenner tumors never are encountered. The second hypothesis is that of Schiller who noted a close resemblance between certain glands of the posterior urethra and the epithelial elements of Brenner tumor. He believed that Brenner tumors arose through a dislocation of similar cells from the primitive wolffian duct. However, although the urinary and the genital anlagen develop in close proximity to each other, direct connection has rarely been proved to exist within the ovary. The third hypothesis, namely, that the teratomatous origin, is based on the fact that about a third of Brenner tumors occur in the walls of mucinous cysts of the ovary. In a few cases there has been uterine bleeding at irregular intervals but the tumor is not known to produce any hormones which could be held accountable for this symptom. In 5 cases reported in the literature, a Brenner tumor complicated pregnancy. Ascites is produced occasionally. In 1 case hydrothorax completed the picture of Meigs' syndrome. One should never forget that certain benign ovarian tumors can imitate malignancy in this way. A surgical diagnosis on macroscopic examination of the specimen is rarely possible, but the general gross characteristics of Brenner tumor are those of a benign neoplasm. Microscopically, the chief danger is in mistaking Brenner tumor for primary or metastatic epithelioma. The predominantly fibrous nature of the growth, the complete absence of mitosis and the peculiar combinations of islands of squamous

cells showing central transitions to columnar elements are the salient points of diagnosis.

In discussing the histogenesis of these tumors Danforth⁵ believes that most authorities agree that they have as their anlagen the cell rests described by Walthard. In addition, the occasional finding of Brenner tumors in the walls of pseudomucinous cystadenomas of the ovary, and the more frequent finding of pseudomucinous epithelium in Brenner tumors and Walthard rests, have been cited as indications that at least a portion of the pseudomucinous cystadenomas may have their origin in Brenner tumors. If these inferences are true, one might expect to find certain cytologic as well as architectural features common to all 3 structures. The Walthard rest is a small collection of cells which may be found in the cortex or hilus of the ovary, or beneath the serosa or within the mesosalpinx of the fallopian tube. The rests are of 2 types, the solid and the cystic. In the cystic type the central portion of the rest is occupied by a clear space which is said to contain mucin or pseudomucin, colloid, or mixed material. The cystic cavity is ordinarily lined by from 1 to 10 or more layers of cells. The origin of the Walthard rests is obscure. They are stated variously to arise from the celomic epithelium of the developing embryo, from an inflammatory reaction of the peritoneum, from the germinal epithelium and from numerous other structures. The first named is probably most widely accepted. With an oil immersion lens, the most striking feature of the cells is the nucleus, which is not simply round or ovoid, as it has been described, but in addition is seen to be marked or "tagged" in a specific manner. This marking consists of a tiny groove or fold which traverses the long axis of the nucleus in such a manner as to remind one of a coffee bean, or a kernel of puffed wheat. In many of the rests this characteristic marking is present in approximately 9 of 10 nuclei, being evident as a straight, bold line through the long axis, dividing the

nucleus into 2 almost equal halves. As nearly as can be ascertained, this basic pattern is characteristic of one of the flat surfaces of a healthy, non-degenerate nucleus. In these instances the nuclear material is seen to be very finely granular and to contain, generally, 1 or 2 nucleoli. When 2 nucleoli are present 1 usually appears upon either side of the dividing groove. Walthard rests and Brenner tumors bear a strong resemblance to one another, not only in intrinsic architecture, but also, and more especially, in nuclear detail. The basic nuclear type of each is readily identifiable by the presence of a median groove or fold. The deviations from this basic type are described. This nuclear peculiarity is sufficiently constant to be regarded as a fundamental characteristic of both the Walthard rest and the Brenner tumor. Furthermore, its presence in structures appears to strengthen the hypothesis that they may be derived from the same tissue. The presence of the basic nuclear type in many pseudomucinous cystadenomas is of interest in considering the possibility that a small proportion of these tumors may be derived from Brenner cell rests.

ADRENAL REST TUMOR. This rare type of tumor is frequently but not always associated with masculinization. Greene and Lapp⁹ call attention to the fact that embryologically the ovaries, testes and adrenal cortex have a common origin, being derived from the celomic epithelium of the urogenital fold. This urogenital fold in later embryonic life divides to form the adrenal cortex and the gonads. It is not without possibility, therefore, that misplacement of the cells occasionally occurs in the ovary from the neighboring adrenal anlage. The mere presence of adrenal tissue in an aberrant site does not necessarily mean that the clinical signs of masculinization will always be present. The tendency for aberrant adrenal tissue to become neoplastic cannot be doubted, however. From the recorded cases, no correlation appears to exist between the age of the patient and the occurrence of

the tumor. The tumor has been observed at various ages from 3 to 65. The tumor is generally unilateral, varying in size from several millimeters to 3 or 4 cm., occasionally slightly larger. It is most often noted in the hilar region of the ovary, although it may involve the whole ovary by contiguity. It is a solid tumor, often encapsulated, rarely becoming cystic, occasionally showing small areas of hemorrhage and necrosis on cut surface. The external surface is usually smooth. The tumor is generally of soft consistency, and has a characteristic yellow color, resembling closely its analogue, the adrenal. It is probable that most of these tumors are benign, as is true of comparable lesions of the adrenal. Excision of the tumor frequently produces moderate to complete regression of all signs and symptoms. The voice changes, however, usually are permanent. If the hirsutism does not disappear postoperatively, the presence of additional adrenal cortical tissue in other aberrant locations is a possibility. It is interesting to note the sex hormone produced by the adrenal rest cell produces sex characteristics and changes not of the same but of the opposite sex. In the male, the adrenal rest tumor is known to produce a female type of adiposity, breast hypertrophy, at times with lactation, testicular atrophy and loss of libido.

In reviewing 1 case of their own and 13 cases of this type reported in the literature, Kepler, Dockerty and Priestley¹⁶ found that hirsutism was present in all 14 cases and varied in duration from 9 months to 13 years. A masculine pattern of distribution of hair usually was noted. This was emphasized in 4 instances by an observed recession of the hairline along the forehead, with the notation that the patients were partially bald. In 10 of the 14 cases the habitus was described as being of masculine type with pronounced development of the muscles of the shoulder girdle. In 8 of the 14 cases there was a definite and sometimes pronounced gain in body weight. Moreover, in the majority of the observed instances the obesity

was more or less localized to the face, cervical region and pectoral girdle. Blood pressure readings were recorded in 6 of the 12 cases that were reported more recently. In 4 of these cases, hypertension was present with systolic pressures in excess of 140 mm. or diastolic pressures of more than 90 mm. of mercury. In the case they reported, postoperative readings of the arterial blood pressure indicated a moderate drop in both systolic and diastolic pressures from distinctly high preoperative levels. This finding indicates that in some way the ovarian tumor was responsible for the observed arterial hypertension. Diabetes or a diabetic tendency was of relatively frequent occurrence. Hematologic observations suggesting polycythemia vera were unusually common in this group. Size has varied from nodules of microscopic dimensions to masses measuring 16 x 14 x 9 cm. The tumors were for the most part yellowish in color and encapsulation was the rule in practically all recorded instances. Microscopically, the tumors were composed of large, pale, polyhedral, epithelial-like cells disposed in cords and anastomosing strands. Large amounts of intracellular lipid material were noted in nearly all cases and intercellular glycogen was occasionally found to be present. Like arrhenoblastomas, the adrenal-like ovarian tumors tend to masculinize their hosts but the tendency toward the production of a bizarre type of fat distribution, the occurrence of purplish striae, arterial hypertension, polycythemia, etc., relate them more closely to adrenal cortical carcinomas from a functioning standpoint. Histologically, the tumors do not appear to arise through luteinization of preëxisting granulosa cell neoplasms and an origin from adrenal cortical rests seems more logical than does a derivation from corpora lutea. An origin by way of teratomatous development likewise remains unproved. Some of the tumors appear to be cytologically malignant and in 1 instance death resulted from metastasis.

STRUMA OVARII. The presence of thyroid tissue in an ovarian tumor is not

common. Gusberg and Danforth¹⁰ have found only 8 cases over a 20 year period at the Sloane Hospital, representing 2.7 % of 297 ovarian teratomata of all types. Of the 8 cases which they report, 7 presented the usual array of clinical symptoms and signs which ordinarily accompany benign ovarian tumors; intermittent abdominal pain, painless pelvic or abdominal masses, and, in 1 instance, acute abdominal pain due to torsion of the pedicle. In these cases, the presence of thyroid tissue is of pathologic interest only. The cystic tumors were generally multilocular, and 3 of the 7 contained epidermoid elements characteristic of the ordinary dermoid cyst. Microscopically, none of these cases showed evidence of hyperplastic changes in the thyroid tissue. The eighth case, however, presented a symptom complex of an unusual nature, the diagnostic and therapeutic implications of which are deemed significant. In this case a huge struma colli, a large struma ovarii and evident thyrotoxicosis were coëxistent. It is unfortunate that the possibility of ovarian struma was not considered and the preliminary work-up carried out with this in mind. That struma ovarii is capable of producing or at least contributing to thyrotoxicosis, is established. It is this ability which distinguished the ovarian struma among all ovarian neoplasms, and removed it from the category of the simple pathologic curiosity. It should be emphasized that cases of functioning ovarian struma may easily escape diagnosis, either through failure to appreciate mild hyperthyroid symptoms, or through chance avoidance of thyroid tissue in the sectioning of large ovarian teratomata. It is of interest that in most of the cases in which such data are available, the ovarian sections have failed to show the presumed microscopic stigmata of hyperthyroidism, despite amelioration of symptoms by removal of the tumor. It would appear reasonable to consider the ovarian struma as a functioning part of the total thyroid tissue of the body, which may react to iodine lack,

to the thyrotropic hormone, or to other stimuli in a manner similar to that of the thyroid gland itself. If this hypothesis is tenable, one might then consider the removal of an ovarian struma as tantamount to subtotal ablation of the thyroid gland.

TERATOMA. In reporting 1 of these interesting tumors, Curtis⁴ states that, although encountered in other organs and tissues of the body, teratoma is predominantly an ovarian tumor and it is here that it is found in most developed and characteristic form. It is usually a compact, solid, rapidly growing neoplasm composed of tissues which are frequently wholly undifferentiated, corresponding to various stages of fetal development and revealing only isolated tendency to more completely developed organ-like formation. The material entering into the structure of dermoids and teratoma is the same, all 3 germinal layers represented in both; the differences lie apparently in the tissue of origin and in the fact that dermoids are comprised of mature tissues whereas a teratoma consists of embryonal elements. The great characteristic of teratoma is the loss of proportion in its structure. There is topographic confusion. Opinions differ in judgment of the malignancy of teratoma; it is a growth characterized by capacity for great proliferation, not ascribable to carcinomatous or sarcomatous degeneration but an inherent property of the cells which constitute it. Therefore, it is to be regarded as potentially malignant in all cases, unquestionably malignant in most instances. The tumor is usually nodular or lobulated, often with projecting cysts, of astonishingly large size for so malignant a neoplasm (due to rapid growth?), often the size of a man's head. It may be spherical, ovoid or of ovarian shape, is usually pedunculated, of varied color—gray, yellow, red, blue, brown. The consistency is usually soft, but many are firm. A capsule is usually present but may not be clearly defined. Perforation of the capsule is frequent, but by no means usual. Microscopically, the picture is that of chaos. The tissues are

mostly fetal in character, but not entirely and invariably so. It is characteristic for well-differentiated tissues to lie immediately adjacent to lawless and wild areas of potentially or definitely malignant epithelial, endothelial, or sarcomatous pattern. Teratoma is a tumor of childhood and early maturity, mostly before the age of 35. Not more than 5 recorded cases have been found after the menopause. The etiology remains unsolved. The growth must be derived from a cell that can produce all 3 embryonic layers. Only 2 theories of origin are prevalent: that isolated blastomeres are the source of these trigerminal growths or that they arise from primitive unfertilized ova.

In presenting 32 collected cases, in which the outcome is known, including 2 cases of his own, Smeltzer²⁵ found that 21 (65%) are dead while the other 35% have survived from 1 to 10 years. It was noted that the tumors were practically always pedunculated and the surgical treatment carried out was simple excision of the pedunculated tumor or adnexa on that side. The high rate of recurrence of tumor and death indicates that a more extensive surgical procedure is indicated. These tumors are usually well encapsulated. There is ordinarily a well-formed pedicle; seldom is there any more than very fine adhesions to the surrounding organs. The findings of extension or gross metastasis is rare at the time of the first operation. To all appearances when the abdomen is first opened the operator is dealing with a benign tumor, or at least one of very low malignancy. These observations, added to the fact that the patient is young, make it very difficult to proceed with radical surgery. The pedunculated tumor recurs at the site of the original lesion. Later it spreads from this area by extension as well as metastasis. However, metastasis seems to be a late manifestation. This observation further emphasizes the need for wider excision at the time of removal of the primary growth. The effect of Roentgen ray therapy upon this type of tumor is debatable. These

cases should be given Roentgen ray in therapeutic amounts immediately after the primary operation. How effective Roentgen ray will be in preventing a recurrence he does not know but he does not believe it is beneficial after recurrences are present. He believes that more emphasis should be placed upon a wider excision of the original growth. This means removal of all the pelvic viscera as widely as the operator's experience allows him to dissect. The operation should then be followed by Roentgen ray in the amount compatible with the patient's age and size. Girls with teratoma should be treated in a manner similar to adult women with carcinoma of the ovary.

CARCINOMA. All are agreed that one of the most effective weapons we have against any cancer is prevention or at least early detection and removal. Ovarian cancer is notoriously "silent" in its early stages and we all have had the experience of Crossen³ who states that patient after patient is seen with an extensive growth of long duration but with only a short period of local symptoms. The first visit to the physician shows a large advancing carcinoma causing only such minor disturbances that the patient hardly notices them. Thinking back, when questioned, she recalls that the abdomen has been a little larger for a year or so or that there was bloating or some frequency of urination, but only in the last few weeks was there enough disturbance to make her feel that perhaps an examination was advisable. He believes the greatest contribution the physician can make toward the lessening of these fatalities is the practical application in his daily work of 2 rules: (1) removal of the involuting ovaries whenever the abdomen is opened at any age and under circumstances which permit such removal; (2) insistence on regular periodic pelvic examination of patients who ask us to assume responsibility in regard to their health. The menopause (complete cessation of menstruation) occurs usually between the ages of 44 and 47, with exceptions somewhat below and

above these limits. The climacteric (gradual involution of the ovaries) begins 2 or 3 years before the menopause and extends some years after it. Though there is considerable individual variation as to age of onset of ovarian involution, a reasonable rule would be to apply this safety measure in all abdominal operations when the patient has reached the neighborhood of the age of 42. The involuting ovaries have fulfilled their reproductive and endocrine functions. They are no longer an important part of the economy but vestigial structures which carry a special tendency toward cancer—and toward a particularly dangerous form of cancer, in that it develops to an incurable stage without warning symptoms. The realization of the necessity of adopting this safety measure has been obscured and delayed by the various arguments for retention of an ovary. In the first place, there is the excellent surgical rule to do no more operating than necessary—to remove no structure without a definite reason of sufficient importance to justify the additional operative risk and any probable upset in physiology. Again, the great importance of the ovaries during the span of their physiologic activity projects about them a halo which tends to obscure the fact that they are only temporary organs that cease to function after a certain period. Again, there is the desire to lessen the hot flushes and other disturbances of ovarian cessation by preserving ovarian tissue even though it is functioning only partially. Still again there is the natural desire of the patient that an ovary be saved if practicable. These are all valid arguments and have long influenced decisions, but through careful observation and bitter experiences he has come to realize that the danger of cancer development in the involuting ovary outweighs all these arguments for leaving an ovary in an abdominal operation in the age of involution. In the period from 40 to 60 years of age pelvic examination is advisable every 6 months instead of once yearly, as may suffice before and after that period.

Symptomless onset and symptomless progress to incurability constitute the natural history of ovarian carcinoma. Earlier discovery is due to some incidental associated condition or to a pelvic check-up examination. The aggregate of deaths from this cause is large, much larger than is generally appreciated. To the known cases must be added the unrecognized ones with death certificate designations of "ascites" and "abdominal cancer." A large proportion of the cases of general abdominal carcinosis originate in an ovary. The silent character of the onset and progress of ovarian cancer seals the doom of patients with this condition unless measures that are really effective against the serious difficulties of the situation are put into practice.

In reviewing the end-results in a series of 138 patients with ovarian carcinoma operated upon at Roosevelt Hospital in New York, Taylor and Greeley²⁶ found an "absolute" 5 year cure rate of 15.2%, when all cases are considered and the lost cases are counted as dead. It is well recognized that the gross extent of the ovarian carcinoma at the time the operation is undertaken is the most important single consideration in prognosis. If one defines an operable case as one in which all cancer was apparently removed, the operability of this series was 31%. The known cures in this operable group were 45.5%, while if the many cases untraced for the necessary 5 years, but free of disease when last seen, be excluded, the 5 year cure rate in the operable group becomes 58.8%. The other side of the picture is as gloomy as this is encouraging, for of the 94 cases in which malignant tissue was left behind at the conclusion of the operation or which was recurrent, only 2 have survived the 5 year period. The cancers of the ovary are distinguished by the diversity of their cells of origin, and evidence has been presented in the literature to show that certain of the special types, such as the granulosa cell tumors and dysgerminomas, have a relatively favorable prognosis. The grade of

malignancy in ovarian cancer is of enormous prognostic significance. The classification of 88 cases of ovarian adenocarcinoma indicates only 2 cures among 52 undifferentiated types, whereas among 36 so-called Grade 1 cases there were 12 cures. In a series of cystadenomas and adenocarcinomas of the ovary, the morphologic transition from the benign to the malignant takes place almost imperceptibly. No pathologist has had the experience to place the line of division exactly. Difference of opinion as to what histologic features justify the classification of some of these borderline tumors as benign or malignant is probably the most important variable governing the reported figures on cures of ovarian cancer. A slight change in conception of the morphologic criteria of malignancy in the cystic tumors of the ovary appears to them largely to explain the change in their own figures from the 8.3 cure rate of their 1934 report to the 15.2% noted in the present article. It does not appear that radiation, as given at the Roosevelt Hospital, has materially affected the average duration of life in cases of ovarian cancer.

The seriousness of ovarian malignancy has been given considerable thought by Goodall⁸ who feels that there is no clinical criterion by which one can distinguish a non-malignant from a malignant ovarian growth. Especially is this true while the malignant growth is still limited to the ovary and encapsulated. Yet this is the only period in which malignancy can be dealt with satisfactorily. Ovarian new-growths are unfortunately singularly free from symptoms. Even the menstrual cycles and characters are seldom disturbed. The advent of pain, or of free fluid in the abdomen are too often the heralds of in-eradicable extensions. When upon opening the abdomen, the case presents a definite peritoneal involvement, he knows of no rule which can be laid down to guide the surgeon. Only experience can be a safe judge. In these circumstances both ovaries are generally involved, one higher up, and the other bound down by

peritoneal extensions in the pouch of Douglas. When, however, the ovarian growths can be removed without too much risk, even at the expense of considerable difficulty, it is thought that the period of life is lengthened. This is a debatable point, for no one can state what the tempo of that tumor was before operation, and what will be its rate of growth afterward. When the case is operable it is his policy to remove both ovaries whenever there is undoubted malignancy of one of them. This is not so radical a procedure as one might suspect. Most of these patients are in their late thirties or older, and spaying, as a rule, does not involve any grave consequences. It would seem that deep Roentgen ray application should find in malignant ovarian disease a field of great usefulness, but he has found deep Roentgen ray therapy singularly inefficient in the cure or alleviation of cases of peritoneal involvement. It is his conviction that Roentgen rays not only do not improve, but have almost invariably broken down the patient's health and hastened death. He concludes that the only hope for a patient suffering from incipient malignancy of the ovaries lies in early extirpation, before the disease has spread beyond the confines of the ovary. This will necessitate exploratory operation upon any suspicious tumor of the ovaries. Our attitude toward ovarian newgrowths must be somewhat akin to the position we adopt toward tumors of the breast.

A somewhat more optimistic note as to the value of Roentgen therapy is expressed by Walter, Bachman and Harris²⁷ who have reviewed the literature on postoperative radiation therapy of ovarian carcinoma and present 124 additional cases treated by surgery alone or by surgery and postoperative radiation. They believe that failure to classify the cases according to stage of progression has made evaluation of the early results of therapy difficult. The classification they employ groups the cases into 4 stages: (1) a primary unilateral and completely removable tumor with

no visible metastases, (2) the presence of local metastases which are completely removable with the primary tumor, (3) the presence of local metastases which are only partially removable with the primary tumor, and (4) cases with advanced carcinomatosis peritonei for which exploratory celiotomy or removal of large tumor masses are diagnostic and palliative measures. In Stage 1 and 2 cases reported in the literature the supplementary use of radiation therapy did not significantly alter the five year survival rate, but the increased number of five year survivals of Stage 3 cases in the irradiated group appears to be of statistical significance. The prognosis in Stage 4 is extremely grave.

Further value of Roentgen therapy is suggested by Parks²⁴ who reports 3 cases of papillary cystadenocarcinoma of the ovary, inoperable because of massive infiltration into the surrounding structures, which were apparently made operable by deep Roentgen ray therapy. In all 3 cases the neoplasm was limited to the abdominal cavity. The Roentgen ray profoundly affected the gross appearance of the tumors, but caused very little change in the microscopic picture. Two of the patients are alive after 8 and 12 years, respectively; the third succumbed after 5 years of an apparently unrelated carcinoma. Deep Roentgen ray therapy in the 3 cases reported caused the papillary excrescences and transplants to shrink and the entire mass to diminish in size, thus making operation easier. This study indicates that it is unwise to persist in doing a difficult primary operation. In some cases of massive infiltration, it may be safer to take a biopsy, close the abdomen, give deep Roentgen ray and perform a second operation at a later time.

ADENOACANTHOMA. A rare type of cancer, composed of both glandular and squamous elements is termed adenoacanthoma. In reporting 2 such lesions in the ovary, Melody, Faulkner and Stone²⁸ were unable to find another instance of primary adenoacanthoma of the ovary in the literature. They state that the histo-

genesis of this type of lesion is much disputed. Some believe that the "epidermization" seen in fundal adenoacanthoma is the result of metaplasia of the cylindrical to the squamous type of epithelium. A study of cervical biopsy material reveals the very great frequency of metaplasia of the normal columnar to the squamous type of epithelium, in inflammatory lesions of the cervix uteri. Moreover, it is recognized that the germinal epithelium covering ovaries which are involved in chronic perioöphoritis occasionally does undergo squamous metaplasia. Then, there is the condition known as cystic fibrosis of the pancreas in which, due to the impaired absorption of fat-soluble vitamin A, there is a pronounced squamous metaplasia of the bronchial mucosa. It would appear, therefore, that squamous metaplasia is really of rather frequent occurrence in a wide variety of pathologic processes, running the gamut from chronic inflammatory lesions, and a vitamin deficiency state, to frank neoplasms. It seems entirely tenable, therefore, to assume that the squamous elements in the ovarian adenoacanthomas are the result of the ubiquitous process of metaplasia. Another plausible hypothesis for the presence of squamous epithelium in ovarian carcinoma is the supposition that such may result from Walthard's islands which are thought to result from the "invaginations of celomic epithelium." The manifold possibilities of celomic epithelium on differentiation are well known. It well may be that the origin of the squamous elements in ovarian acanthoma is identical with the generally accepted theory of histogenesis of Brenner tumor, *viz.*, Walthard's cell rests. The authors, however, incline to the view that the squamatization seen in the tumors described is the result of metaplasia.

KRUKENBERG TUMOR. In presenting a study of 44 cases of Krukenberg tumors from the Mayo Clinic, Leffel, Masson and Dockerty¹⁸ state that most workers agree on the fact that this is usually a secondary tumor and no description of it has surpassed that originally given by Kruken-

berg. He stated that the tumor was of moderate size; it was nodular yet it possessed a smooth surface. Adhesions were usually absent in such cases but ascites was often found. The tumor was usually bilateral, usually solid, occasionally cystic and the general form of the normal ovary was usually maintained. The cut surface had a myxomatous appearance. Microscopically, Krukenberg described "signet-ring" cells surrounded by a fibrous stroma. He recognized the picture as one of undoubted malignancy and considered the lesion as primary in the ovaries. However, there is as yet no general agreement as to what constitutes a Krukenberg tumor; as to how metastasis occurs; as to whether a primary ovarian carcinoma may be impossible to distinguish from a Krukenberg tumor; as to whether the metastasis is in any way selective; or on how the lesion should be treated. They feel that these tumors should be described, like other metastatic tumors, merely as metastatic carcinoma of the ovary. Realizing, however, that the term "Krukenberg's tumor" will continue to flourish in medical literature, they contend that it should include all metastatic adenocarcinomata of the ovary. There is no real difference between metastatic adenocarcinoma of the ovary and metastatic adeno-"colloid" carcinoma of the ovary, save for the presence of mucus in the latter. However, if this difference were overlooked, not even the remotest reason would ever exist to use the term "Krukenberg's tumor." Metastasis may occur in 4 ways: (1) spread by peritoneal sedimentation, (2) spread by lymphatic channels which must include retrograde spread, (3) extension by continuity, and (4) spread by way of the blood stream. The treatment of this tumor depends on many factors, chief of which are the patient's general condition and the extent of the growth. Given a patient who is a poor surgical risk or one showing evidence of extensive metastasis, surgical treatment is contraindicated, save for the relief of an obstruction of the gastro-intestinal tract. Given a patient

who is a good surgical risk without extensive carcinomatosis, the uterus, tubes and ovaries should be removed. This procedure is justified for 3 reasons: (1) the pathologist's diagnosis may be wrong, (2) the remaining days or months which the patient has to live will be more comfortable without the presence of a huge pelvic mass and abundant ascites, (3) the increased mortality and morbidity associated with this procedure are not great enough to warrant not attempting to give the patient the comfort that would result from such a procedure. Circumstances may justify leaving the uterus intact but an apparently atrophic ovary should never be left intact. Every surgeon cannot have a pathologist at his side, particularly one who is experienced with the diagnosis of fresh tissue. A good general rule, in this case, is to remove both ovaries when a solid tumor is found involving either one, particularly if the patient is past or near the menopause. The treatment of the primary lesion usually depends upon the site. All things being equal, the surgeon is justified in being more radical when the primary lesion is in the colon than when it is elsewhere, because of the frequent relatively low grade of these lesions.

FIBROMA. Although ovarian fibroma is a benign lesion, it is the second most common solid tumor of the ovary. In a study of 283 cases from the records of the Mayo Clinic, Dockerty and Masson⁷ never found a fibroma before puberty, which they believe indicates an origin possibly based on a desmoplastic reaction to the hemorrhage of ovulation or ovarian endometriosis. Fibroma did not produce any specific diagnostic symptoms and rarely was it possible for the clinician to go further than to diagnose an ovarian tumor. Abdominal ascites (51 cases) and hydrothorax (2 cases—syndrome of Meigs) suggested the existence of a malignant process but the patients never presented the picture of cachexia. The complications of these tumors were chiefly those associated with twisting of the pedicle of the tumor—a phenomenon which rarely

occurred until the tumor outgrew the confines of the true pelvis. Pathologically, most of the tumors were solid throughout, white and usually invested by a smooth capsule free from adhesions. Many of the tumors were edematous and a number of these had undergone degenerative changes with central cysts or "geodes." The common denominator relating to both ascites and formation of cysts was a weeping edema effected through partial obstruction of the venous return. In 90% of cases the tumor was unilateral. Bilateral fibroma-like tumors sometimes proved to be metastatic tumors of the Krukenberg type with the primary neoplasm most frequently in the stomach. A yellowish color suggested theca cell tumor, especially in cases in which the uterus was large and postmenopausal bleeding was noted clinically. In others the yellow color resulted from fatty metamorphosis. A grayish brown color and firm consistency were noted in several tumors that later proved to be of the Brenner type. A brownish color and soft consistency indicated malignant change, which occurred in 1% of the tumors studied. Degenerative changes, such as fatty, fibrous hyaline and calcareous, took positions of importance secondary to the phenomenon of intercellular edema, which correlated more universally than did gross edema and formation of cysts with the clinical production of ascites.

In an effort to explain how fluid gets into the pleural cavity in some cases of ovarian fibroma (Meigs' syndrome), some interesting studies were made by Meigs, Armstrong and Hamilton.²² In 2 patients 2 cc. of sterile India ink was injected in the abdomen, and chest taps performed later. In each instance the fluid in the chest showed the same concentration of India ink as in the abdomen. That the India ink did not arrive in the pleural fluid by way of the blood stream was considered established by study of the blood shortly after injection of the abdomen with India ink. In the 2 patients the blood showed no evidence of India ink in the

leukocytes on microscopic examination. This experiment is suggestive evidence that the abdominal fluid in these patients arrives in the chest by the same pathway as the India ink, or *vice versa*. The probability is that the pathways are lymphatics, first through the interstices of the cells under the diaphragm, thence to the supra-diaphragmatic lymphatics and thence into the chest. The passage of particulate carbon is of considerable interest when considered with relevance to the mechanism of hydrothorax in conjunction with ascites in certain conditions other than Meigs' syndrome. Statistical considerations enhance this interest for Meigs' syndrome is rare in comparison with ovarian fibromas with ascites alone. So, likewise is hydrothorax and ascites rare in comparison with Laennec's cirrhosis with ascites alone. It may be that hydrothorax is found in conjunction with ovarian fibroma or portal hypertension in those few patients who also have sufficient congenital mechanical communication between peritoneum and

pleura. Extension of this hypothesis leads to investigation of the possibility of such communication in conditions where the basis of fluid in both cavities is more readily explicable, *e. g.*, cardiac decompensation and metastatic carcinoma. Initial experiments in this investigation have been completed. In 2 patients, 1 with carcinomatosis, the other with Laennec's cirrhosis, particulate carbon injected into the pleural fluid failed to attain the peritoneal fluid within 24 hours. At first glance, this result might be taken to indicate that no communication exists in these instances. However, it should be noted that in 2 cases of Meigs' syndrome, only communication from abdomen to chest, not from chest to abdomen, has been demonstrated. It remains for joint investigation by thoracic surgeons and others to demonstrate the presence of diaphragmatic perforations of small or large size, the presence of the rarely reported pleuroperitoneal tubes, and to determine the direction and degree of penetrability of diaphragmatic lymphatics.

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PHYSIOLOGY

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Postburn Azotemia, Its Significance as Criterion of the Severity of Thermal Injury and of the Efficacy of Fluid Therapy.

OTTO ROSENTHAL, M.D., and MILES D. MCCARTHY, PH.D. (Harrison Dept. of Surg. Res., Univ. of Penna.). Non-protein nitrogen, urea nitrogen and amino nitrogen were determined in the plasma of rats subjected to standard burns of known lethality (M. D. McCarthy, *J. Lab. and Clin. Med.*, 30, 1027, 1945). The difference between non-protein nitrogen and the sum of urea and amino nitrogen was designated as undetermined nitrogen.

Following the scalding, 1 group of rats received no treatment while others were transfused with physiological saline solution, normal rat plasma, human albumin or gelatin.

The increases in the undetermined nitrogen and amino nitrogen of plasma reflected the severity of burn whereas the urea levels did not. The proportionally largest increase occurred in the undetermined nitrogen fraction, the levels of which furnished a sensitive criterion of the severity of burn. This finding is in accord with the experience in man (J. Walker, Jr., *AM. J. MED. SCI.*, 209, 413, 1945).

The degree of permanent reduction of the increased amino nitrogen and especially of the undetermined nitrogen levels of plasma provided an index of the efficacy of fluid replacement therapy, whereas the response of the urea nitrogen levels was too slow and too irregular to be of use for this purpose.

The effectiveness of fluid replacement therapy in permanently reducing the elevated undetermined nitrogen levels was not clearly related to the relief of hemocentration. It is suggested that a renewed rise of the undetermined plasma nitrogen levels in spite of nearly normal

hematocrit values is an indication of a returning tissue anoxia due to a diminution of the actively circulating rather than of the total blood volume.

Cerebral Blood Flow and Metabolism in Patients With Increased Intracranial Pressure. HENRY A. SHENKIN, M.D., SEYMOUR S. KETY, M.D., FRANCIS C. GRANT, M.D., and CARL F. SCHMIDT, M.D. (Depts. of Pharmacol. and Neurosurg., Univ. of Penna.). Observations on the cerebral blood flow utilizing the nitrous oxide method of Kety and Schmidt, cerebral metabolism, cerebrospinal fluid pressure and blood gas concentrations were made on 12 patients, 9 of whom had increased intracranial pressure.

A reduction in cerebral blood flow is well correlated with increased intracranial pressure due to brain tumor. The average cerebral blood flow in the 9 patients with increased intracranial pressure was 52.7 cc. per 100 gm. of brain per minute. The normal cerebral blood flow has been found to be an average of 70 cc./100 gm./min. determined by the same technique.

Cerebral oxygen consumption was decreased by increased intracranial pressure but was not correlated to the degree of increased pressure. The average oxygen consumption of patients with increased intracranial pressure was 3.7 cc./100 gm./min., as compared with an average normal of 4.3 cc./100 gm./min. Alteration in the states of consciousness of the patients seemed better related to the decrease in cerebral metabolic activity than to the decrease in cerebral blood flow.

The mean arterial blood pressure (aver. 115 mm. of mercury) was unequivocally increased in all patients in this series with increased intracranial pressure and cor-

related well with the decrease in cerebral blood flow.

In those patients with high increased intracranial pressure and markedly reduced cerebral blood flow, rapid reduction of the increased intracranial pressure by ventricular drainage or 50% glucose solution given intravenously did not improve the cerebral blood flow. In patients with moderately increased intracranial pressure and moderately decreased cerebral blood flow, 150 cc. of 50% glucose solution given intravenously markedly increased cerebral blood flow. Ventricular drainage has not been applied, as yet, to patients in the latter situation.

Biological Precursors of Uric Acid Carbon. JOHN C. SONNE and JOHN M. BUCHANAN, PH.D. (Dept. of Physiol. Chem., Univ. of Penna.). A study of the biological precursors of uric acid carbon has been made by administering to pigeons compounds labeled with C^{13} (*i. e.*, isotopic $NaHCO_3$, carboxyl-labeled acetate, lactate, and glycine and lactate labeled in the α or β positions). Uric acid was isolated from the excreta, purified and degraded by chemical methods in such a manner that each of its 5 carbon atoms could be obtained separately. It was found that CO_2 is the precursor of carbon 6 of uric acid; the carboxyl carbon of acetate is the precursor of carbons 2 and 8; and the carboxyl carbon of glycine is the precursor of carbon 4. The carbon atoms of lactate were also incorporated into uric acid but in a smaller order of magnitude than the carbons of CO_2 , acetate and glycine. Carbons 4 and 5 of uric acid were derived from the carboxyl and α (or β) carbons of lactate respectively. It is thought that glycine may be formed from lactate by reactions similar to the conversion of serine to glycine (D. Shemin, *J. Biol. Chem.*, 162, 297, 1946).

In a supplementary experiment it was shown that the carboxyl carbon of acetate is not a direct precursor of urea carbon in the rat.

From the data presented, the conclusions were drawn that glycine and acetate are not interconvertible in the metabolism of the pigeon and that the precursors of urea carbon in the rat and of ureide carbon in the pigeon are different.

Permeability Methods for the Recognition of Abnormal Erythrocytes. M. H. JACOBS, PH.D., W. J. BROWN, M.D., DOROTHY R. STEWART, PH.D., and L. J. KIMMELMAN (Dept. of Physiol., Univ. of Penna.). In the usual clinical fragility test with hypotonic salt solutions conditions of osmotic equilibrium are observed. These are strongly influenced by pH, temperature and other factors, and require an inconveniently long time for their attainment. In the present method, which utilizes the characteristic permeability of erythrocytes to solutes such as glycerol and thiourea, osmotic hemolysis also occurs, but the attainment of an equilibrium condition is unnecessary, and a time of 3 minutes usually suffices for a test. The results are also more reproducible and they demonstrate abnormalities more clearly than do those obtained by the older method.

The blood is collected without an anticoagulant in 0.9% NaCl buffered with phosphate at pH 7.4. After suitable adjustment of the opacity of the resulting suspension of erythrocytes, it is mixed with the hemolytic solution in such proportions that hemolysis takes place in one or the other of the following solutions: (1) 0.3 M glycerol, 3 parts; 0.9% NaCl, 2 parts; or (2) 0.3 M thiourea, 4 parts; 0.9% NaCl, 1 part. The solution containing glycerol must be free from copper. The course of hemolysis is preferably followed by a photoelectric method which yields a permanent photographic record for analysis. In an alternative method hemolysis is stopped by osmotic means at selected points, and the hemoglobin previously liberated by the cells is then

measured. In a much simplified method the time required to reach a chosen degree of hemolysis is determined.

When studied by any of these methods, normal blood shows certain relatively constant properties, and departures from the latter are easily recognized. Characteris-

tic retardations of hemolysis have been found in primary pernicious anemia, sickle-cell anemia, obstructive jaundice, and advanced carcinoma; equally characteristic accelerations have been obtained in hemolytic anemia and during the aging of bank blood.

BOOK REVIEWS AND NOTICES

SHOCK TREATMENTS AND OTHER SOMATIC PROCEDURES IN PSYCHIATRY. By **LOTHAR B. KALINOWSKY, M.D.**, Research Associate in Psychiatry, College of Physicians and Surgeons, Columbia University, and New York State Psychiatric Institute and Hospital; Assistant Neurologist, Neurological Institute of New York; and **PAUL H. HOCH, M.D.**, Assistant Clinical Psychiatrist, New York State Psychiatric Institute and Hospital; Instructor in Psychiatry, College of Physicians and Surgeons, Columbia University. Foreword by **NOLAN D. C. LEWIS, M.D.** Pp. 320. New York: Grune & Stratton, 1946. Price, \$4.50.

Shock treatments have been employed for more than 10 years. The chapters are: Historical Developments; Insulin Shock Therapy; The Convulsive Therapies; Combined Insulin-Convulsive Treatment; Other Somatic Neurological Treatments and Their Relation to Shock Treatments; Prefrontal Lobotomy and Its Relationship to Shock Therapy; Theoretical Considerations.

Insulin shock therapy has not fulfilled early expectations; small doses stimulate the appetite and it is a useful sedative. Metrazol as a convulsive was extensively used for a time, but intravenous administration is difficult and frequent fractures a disadvantage. With electro-shock, the current is under full control and the effect is immediate. Sodium amytal affords sedation and removes inhibitions. Continuous sleep may be produced by the barbiturates with best results obtained in mild excitements. Induction of malaria in paretics has long been in use; such residual symptoms as sometimes persist, may be cleared through shock therapy; possibly penicillin may prove the best agent with which to attack paresis. Re-

frigeration therapy is dangerous and has been abandoned. When shock treatments fail, beneficial effects may be obtained through lobotomy in some schizophrenics, the psychoneuroses and less frequently in involutional depressions; this does not effect a cure, but symptoms may be relieved through removal of the emotional impact.

N. Y.

ESSENTIALS OF NEURO-PSYCHIATRY. By **DAVID OLKON.** Pp. 310. Phila., Lea & Febiger, 1945. Price, \$4.50.

The author has attempted, in a small volume, to cover the many clinical conditions which constitute the field of Psychiatry. He gives brief descriptions of the ordinarily accepted groups of mental and emotional disorders. He has devoted a full chapter to his own personal observations of the capillary bed and brings together his concepts of this experience in the mentally diseased.

The book is well-written and interestingly illustrated. However, it seems to the Reviewer that there is a marked disproportion in the material presented. The earlier chapters on genetics and other factors which the author feels are related to development of the personality would seem to be unnecessary. Emphasis is often misplaced. For instance, the author dismisses the immense problem of homosexuality in 1 paragraph; yet, there are 6 pages on "Transvestitism." His remarks on onanism would not be acceptable to most schools of Psychiatry today. Similar misconceptions and disproportions occur throughout the book.

Also, it seems to the Reviewer that the whole pattern of the book leads to confusion because of no definite integration of parts; its value to the medical student and to the men active in the field of Psychiatry would

seem to be slight and I doubt if it would add very much to the general medical man's concepts of Neuropsychiatry. G. S.

MOTOR DISORDERS IN NERVOUS DISEASES. By E. HERZ and T. J. PUTNAM. Pp. 184. New York: King's Crown Press, 1946. Price, \$3.00.

THE methods of visual teaching used by the Services during this War will have inevitable extension into civilian medical pedagogy. This little book is one illustration of this trend, as it was prepared as a descriptive syllabus for "study, before and after viewing a projection of films" on the various disorders of the motor system. It is a description of disorders of gait, coördination, reflexes and of the mechanisms concerned with eye movements and other disorders of function of the nervous system.

It is illustrated from the films, in part. Needless to say, these are much less effectual than seeing them projected. A number of other diagrams of anatomic nature are included from various sources. The combination of the book and films will undoubtedly provide an interesting introduction to motor disorders. G. G.

AMBULATORY PROCTOLOGY. By ALFRED J. CANTOR, M.D., Associate Proctologist, Kew Gardens General Hospital, Long Island, N. Y. With a Foreword by BEAUMONT S. CORNELL, M.D., Editor, *American Journal of Digestive Diseases*. Pp. 524; 347 special drawings on 275 figures. New York: Hoeber, 1946. Price, \$8.00.

THE wide scope of this book stems from the author's belief that proctology should include all colonic pathology and not simply anorectal lesions. The introductory chapters on clinical anatomy, diagnostic methods, anesthesia and preoperative and postoperative management deal entirely with anorectal disease. Subsequent sections, devoted to specific lesions of the anus and rectum, contain good discussions of etiology and pathology and practical and detailed descriptions of therapeutic operative procedure. The chapter on pruritus is particularly complete and features a favorable description of the author's special therapeutic procedure, tattoo-neurotomy. The remainder of the volume deals in detail with lesions invading the entire colon (colitis, venereal diseases,

diverticulitis, benign and malignant tumors). Here again etiology and pathology are fully discussed and both medical and surgical therapy is presented.

Numerous good illustrations are valuable adjuncts to the descriptions of the pathologic conditions and the operative techniques.

This is a comprehensive and useful text for anyone interested in the diagnosis and treatment of diseases of the colon, rectum and anus. W. F.

PHYSICAL CHEMISTRY FOR PREMEDICAL STUDENTS. By JOHN PAGE AMSDEN, Professor of Chemistry, Dartmouth College. Pp. 298; 53 ills. New York and London: McGraw-Hill (International Chemical Series), 1946. Price, \$3.50.

THE author has written a short, well-organized and readable elementary text on physical chemistry. As the title suggests, the exposition of the subject is with a view towards the requirements of premedical students. There has been a need for properly oriented courses for premedical students, not only in physical chemistry but in elementary organic chemistry as well.

From the present Reviewer's standpoint the volume is marred only by the continued use of time-worn "problems." The solution of problems is essential in the understanding of applied physical chemistry; but the Reviewer has failed to find cogent problems with a definite biologic bias. For example, the question is asked "At what temperature will benzene boil on top of this mountain" (at reduced atmospheric pressure)? How much more interesting and pertinent it would be not only for premedical students, but for all interested in aviation at high altitudes, etc., if this problem were redressed in a form to apply to the gas exchange of man. It is hoped that in a second edition of this little book, the text may be more obviously directed towards the needs of premedical students by the choice of apt applications. D. D.

ADVANCING FRONTS IN CHEMISTRY. Vol. I. High Polymers. Edited by SUMNER B. TWISS, Department of Chemistry, Wayne University. Pp. 196; liberally illustrated. New York: Reinhold, 1945. Price, \$4.00.

THIS short monograph, with papers by 10 individual contributors, is the first of a

series of lectures sponsored by Wayne University under the direction of N. E. Gordon.

The "high polymers" to which the book is largely devoted are rubbers, plastics and fibers. These substances have very large molecular weights and possess high degrees of polymerization (*i. e.*, are composed of some 150 to 10,000 units). Their growing importance in industry is generally recognized. However, it may be well to point out (as one of the contributors has done) that beyond the realm of commerce lie the tissues of plants and animals, and their component structures—proteins, nucleic acids, glycogen, starch and cellulose—are all polymeric. Thus, it is probable that the chemistry of high polymers will develop implications for the biologist and physician; one need merely cite the recent development of fibrin film.

Among the problems dealt with is the phenomenon of aggregation. This is recognized to occur in proteins and glycogen. While biologic applications are only incidental and but lightly touched in the presentation, the monograph will make interesting reading to those concerned with the "advancing fronts in chemistry." D. D.

REHABILITATION. Its Principles and Practice. By JOHN EISELE DAVIS, M.A., Sc.D., Veterans Administration Facility, Perry Point, Md. Pp. 264; 9 charts and 6 tables. New York: Barnes, 1946. Price, \$3.00.

THROUGH the ravages of modern warfare, women and children of all ages as well as men now become its victims, with most of the neuroses continuing to develop for many years after hostilities have ceased. The rehabilitation of those thus handicapped is admirably discussed in the following chapters: Effect of War and Depression; The Psychiatric Approach; Types and Disease Entities; The Psychological Approach; Interest and Effort Theories; Elemental Principles of Mental, Nervous and Physical Reconstruction; Modern Methods; Therapeutic Objectives and Results; Handicraft, Education and Art.

If the psychotic patient is not really dangerous, all possible freedom should be allowed as he engages in work and play activities suited to his physical, intellectual and emotional state. In psychoanalysis the basic thought is that the unconscious mind

exerts a profound influence upon our behavior. In the psychologic approach, the worker deals "not with problem cases, but with persons with problems." Psychologic tests are mostly objective and mostly standardized observation of the subject's behavior. Occupational therapy includes handicraft, creative arts, recreation and education. Psychodramatics are important and interesting; among other advantages are the dressing up which causes the patient to feel important, emotional outlet is provided, identification and re-identification of the individual with other personages, old images and associations are recalled through creative and recreative productions. N. Y.

PATHOLOGY OF TROPICAL DISEASES. By COL. J. E. ASH and SOPHIE SPITZ, M.D. Pp. 350; 941 ills. Phila., Saunders, 1946. Price, \$8.00.

THE morbid changes which characterize many of the so-called Tropical Diseases usually are described briefly and often incompletely in textbooks of Tropical Medicine. Likewise textbooks of pathology usually give little attention to these diseases, if they do not omit consideration of them altogether. Thus this volume fills a great need. The following list of infections and other diseases will serve to indicate the contents of the books: (1) Virus diseases; (2) Rickettsial diseases; (3) Spirochetal diseases; (4) Dysenteries; (5) Bacterial diseases; (6) Fungus infection; (7) Diseases caused by Protozoa of the blood and tissues; (8) Nematodes; (9) Trematodes; (10) Cestodes; (11) Diseases caused by environmental factors; and (12) Deficiency diseases. Each of the diseases is defined briefly, with short sections on epidemiology, clinical features and pathology. A short list of references is given at the end of each section, followed by the plates illustrating the characteristic morbid changes. These include photographs of patients and of gross and microscopic specimens, each well chosen. It is difficult in so short a review to do justice to the excellence of the book. One may find an occasional error in a legend or wish for a somewhat more modern viewpoint in the presentation of pathologic physiology; but these faults shrink into insignificance when the whole is considered. It is difficult to see how the selection of material might have been bet-

tered. The authors have drawn the best from a great wealth of information and presented it in a highly skilful manner. They and their publisher deserve more than congratulations.

H. R.

GROUP PSYCHOTHERAPY: THEORY AND PRACTICE. By J. W. Klapman, M.D., Faculty, Northwestern University Medical School. Pp. 332. New York: Grune & Stratton, 1946.

Group psychotherapy has enlisted new interest during recent years. Though, as the author points out, it is not entirely new, this is the first serious attempt to write a book encompassing the field and formalizing the technique. Every worker in group psychotherapy starts with certain assumptions or premises, most of which are discussed briefly, developing certain theories concerning the technique to be used. The text shows that the author has read extensively in the field and his references are well selected. Case examples handled by different group psychotherapy techniques are cited and are explained as demonstrations of favorable or unfavorable reactions. These various techniques include the psychodrama, reëducation, and group psychotherapy in private psychiatric practice, in the mental hospital, and with problem children. Methods to be used in the organization of groups are also mentioned. The book is easy to read and has a reasonable viewpoint about the uses of Psychotherapy. It should be read by every professional and lay person interested in the "Group," as it is a definite step ahead in the development of literature on the subject.

L. S.

AGNOSIA, APRASIA, APHASIA. Their Value in Cerebral Localization. By J. M. Nielsen, B.S., M.D., F.A.C.P., Assistant Clinical Professor of Medicine (Neurology), University of Southern California. 2nd ed. Pp. 286; 59 ills. New York: Hoeber, 1946. Price, \$5.00.

The first edition of Dr. Nielsen's work, published 10 years ago, aroused considerable interest and also controversy; largely because the conclusions that were tentatively advanced were at decided variance with the accepted, albeit admittedly muddled, concepts of aphasia. Since that time, Dr. Nielsen has continued an exhaustive study

of this field and has had access to a large amount of clinical material. In the present edition, he further elucidates the results of his work on speech disorders and cerebral localization. Of particular interest is the material on the rôle played by the minor hemisphere and interhemispheric shifting of language functions. These important concepts, while not new, have received little attention in previous works on this subject.

The author presents his material in 3 sections: Part 1 contains an historical introduction and a general discussion of agnosia, aphasia and apraxia. Part 2 includes a chapter on methods of examination which will be of help to the clinician. The author points out the pitfalls in the usual methods of testing for the aphasia and describes a variety of tests useful in eliciting the different defects. The bulk of this section is devoted to clinico-pathologic abstracts of 136 important case histories, verified by surgery or autopsy, which illustrate the conclusions reached on the location and interrelationship of language centers. Many of these are cases which were studied personally by the author. He has also drawn heavily on Henschen's detailed monograph. Part 3, which is entitled Appendix, might preferably be read first. It contains brief, clear discussions of the cerebral areas involved and their significance in language and mentation. A complete list of the terms used, with their definitions, is also included in this section.

Dr. Nielsen's proposed nomenclature does not introduce any marked changes, but does do much to clarify and standardize the terminology now in use—something that is badly needed.

This book is recommended to neurologists, psychologists and anyone interested in problems of cerebral localization.

W. H.

EXERCISES IN ELECTROCARDIOGRAPHIC INTERPRETATION. By Louis N. Katz, A.B., M.A., M.D., F.A.C.P., Director of Cardiovascular Research, Michael Reese Hospital, Chicago; Professorial Lecturer in Physiology, University of Chicago. 2nd ed. Pp. 288; 141 ills. Phila.: Lea & Febiger, 1946. Price, \$6.00.

According to the author's Preface, "The revision of this book was undertaken in order to continue to meet this need in terms

of the new electrocardiographic knowledge and terminology incorporated in the revision of the companion book on electrocardiography." This edition preserves the format of the first, with description, interpretation and clinical correlation adjacent to the full page reproduction of the electrocardiogram in each instance. W. J.

CLINICAL LABORATORY DIAGNOSIS. By SAMUEL A. LEVINSON, M.S., M.D., Ph.D., Director of Laboratories, Research and Educational Hospital, Chicago; Professor of Pathology, University of Illinois College of Medicine; and ROBERT F. MACFATE, Ch.E., M.S., Ph.D., Assistant Director of Laboratories, Research and Educational Hospitals; Assistant Professor of Pathology, University of Illinois College of Medicine. 3rd ed. Pp. 971; 192 engravings and 15 plates. Phila.: Lea & Febiger, 1946. Price, \$10.00.

THIS manual covers a rather wider range of subject matter than do most of such manuals and describes at least 1 procedure for each purpose in great detail, step by step. The Reviewer has had no opportunity to test the freedom from typographic errors in these detailed quantitative descriptions, formulas, graphs and tables; but the success of 2 previous editions invites confidence.

Procedures applicable in the field of legal medicine and toxicology, procedures and values appropriate in pediatric practice, a chapter on laboratory study of so-called tropical diseases, a chapter on skin tests,

and 1 on milk and water analysis are included. Especially if the specialized monographs in different fields are not available, will this manual be a useful guide and source of information.

There are many well-chosen illustrations. The field covered, however, is so wide that even this generous allowance cannot, of course, compete with the illustrative material available in a library of special monographs covering various fields. Theoretical considerations are severely condensed and are presented in a number of instances in schematic form. The amount of technique and information condensed here in a single volume is noteworthy. J. A.

NEW BOOKS

Merrilleana. A Selection From the General Writings of ELMER DREW MERRILL, Sc.D., LL.D., Arnold Professor of Botany, Harvard University; former Director, Bureau of Science, Manila; Dean, College of Agriculture, University of California; Director, New York Botanical Garden; Administrator of Botanical Collections, Harvard University, and Director, Arnold Arboretum. *Chronica Botanica*, Vol. 10, No. 3/4. Pp. 393; 24 ills. Waltham, Mass.: The Chronica Botanica Co., 1946. Price, \$4.00.

THOUGH only indirectly connected with Medical Science, this volume contains interesting matter of general biologic interest, written by "the American Linnaeus."

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